Methods in Hormone Research

VOLUME V

Steroidal Activity in Experimental Animals and Man

Part C

METHODS IN HORMONE RESEARCH

Volume I: Chemical Determinations

Volume II: Bioassay

Volume III: Steroidal Activity in Experimental Animals and Man Part A

Volume IV: Steroidal Activity in Experimental Animals and Man Part B

Volume V: Steroidal Activity in Experimental Animals and Man Part C

Methods in Hormone Research

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PREFACE

This volume of *Methods in Hormone Research* is the third devoted to Steroidal Activity in Experimental Animals and Man and is designated Part C. Thus, Volumes III–V deal primarily with a documentation of the classic hormonal activities and related or at times unrelated activities which, for convenience, are designated as nonhormonal activities. Again I quote from the preface of Volume III, "This information is vital for many facets of hormone and steroid research, from the more practical details of fashioning more valuable therapeutic agents to supplying the biological data on the activities of steroids which form the very bases for an understanding of structure-activity relationship and of mechanisms of steroid hormone action."

I am again indebted to loyal, dedicated friends who have kindly labored so long and so well for the completion of this volume. Mrs. Iola Graton has patiently worked with her usual fine spirit in her efficient, able manner to make this work possible. Many members of the staff of Academic Press have been most helpful; and to each I give my thanks.

RALPH I. DORFMAN

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Chapter 1

Effects of Steroids on the Central Nervous System

DIXON M. WOODBURY AND ANTONIA VERNADAKIS

I. Introduction

It is the purpose of this chapter to summarize the available literature on the interrelationships of the steroid hormones and the central nervous system (CNS). Homeostatic regulation in the body is controlled by the nervous and endocrine systems. Much research has been devoted to the physiology, biochemistry, and pharmacology of these two systems separately, but not to their interrelations. Since, however, each system is subject to the regulatory influence of the other, it is evident that the nervous system must influence the endocrine system and vice versa.

Of the two aspects of CNS and steroid hormone interrelations mentioned above, the effect of the steroids on the CNS will be emphasized in this chapter. The other important aspect, namely, the effect of the CNS on steroid hormone secretion, will not be considered here. With regard to the effects of steroids on the CNS, the chapter will include not only the effects produced by exogenously administered hormones, but also the effects of the endogenous hormones either released by the injection of tropic hormones or by stimuli (drugs, cold, etc.) which cause release of endogenous adenohypophyseal tropic hormones, or secreted in the naturally occurring diseases of hyperfunction in man. The alterations produced by hypofunctional states will also be considered.

This chapter will also not include the extensive subject of the relation between sex hormones and behavior. Pertinent reviews of this area, however, have been written by Beach Aronson , Lehrman), and Young

The steroids to be considered in this chapter include the adrenocortical, estrogenic, progestational, and androgenic hormones. The adrenal cortex secretes three general types of steroids. The first type, characterized by cortisol and cortisone, is concerned mainly with regulation of metabolism of carbohydrate, protein, and fat. The second type, characterized by the naturally secreted hormone aldosterone and the synthetic steroid deoxycorticosterone, is concerned mainly with regulation of metabolism of electrolytes. The third type, probably represented by steroids such as 11β-hydroxyandrost-4-ene-3,17-dione, is concerned with androgenic function; its effect on the CNS is similar to that of the male sex steroids. Steroids such as corticosterone and dehydrocorticosterone affect both carbohydrate and electrolyte metabolism. Each type of adrenocortical steroid has a different effect on the CNS, as discussed below.

The estrogens include the naturally occurring steroids, estradiol-17β, estrone, and estriol. They exert regulatory effects on the estrous cycle, control development of secondary sexual characteristics, and influence lipid metabolism. The main naturally occurring progestational steroid is progesterone. Besides an effect on the uterus and secondary sexual characteristics, it also has effects on electrolyte and organic metabolism. Testosterone is the principal androgen produced by the testis. It influences the testis and male secondary sexual characteristics and exerts a marked protein anabolic effect.

The early history of the effects of steroids on the nervous system has been discussed by several authors and hence need not be considered here

adrenocortical insufficiency clearly established his cognizance of the characteristic psychological and neurological alterations produced by this disease. In addition, early observations indicated that the sex steroids had profound psychical and behavioral effects on the CNS. Thus in the nineteenth century the groundwork had been laid for a study of the effects of steroids on the CNS, but no systematic research

in this area was forthcoming until parallel advances in the physiology and biochemistry of the steroids were achieved.

II. Neurophysiological Effects of Steroidal Hypofunction and Hyperfunction

A. Effects on Peripheral Nerve, Reflex Activity, Neuromuscular Function, and Skeletal Muscle

1. Hypofunction

The work of Hartman and Lockwood ( ) demonstrated that the adrenalectomized animal has a decreased ability to support reflex activity and that administration of adrenal cortical extract (ACE) corrects this defect. In adrenalectomized rats maintained on sodium chloride solution, they found that the average fatigue time of the spinal reflex arc was only one-sixth that of the ACE-treated adrenalectomized rats. The various components of the reflex arc were measured by comparative stimulation of the contralateral and ipsilateral sciatic nerve and of the muscle itself. The experimental data suggest that adrenalectomized animals have a defect in transmission at the cord level, at the neuromuscular junction, and in muscle, and that all three sites are equally sensitive to the lack of adrenocortical hormones. As discussed below, this state of affairs is quite unlikely; the defect is probably in the supply of essential substrates to muscle and nerve, as a result of the inadequate circulation in adrenalectomized rats. Subsequent investigations have substantiated this view. For example, Hoagland Hoagland *et al.*), and Slocombe *et al.* demonstrated that conduction time in the central nervous system (as measured by the time for an impulse initiated by tactile and/or electrical stimulation of the foot to reach the cerebral cortex) is significantly decreased (by 18%) in salt-maintained, adrenalectomized rats as compared to intact control rats. The decreased conduction time was found to be centrally determined and could be restored to normal by cortisone. Such data, along with those of Hartman and Lockwood, strongly suggest that adrenocortical insufficiency delays synaptic transmission in the central nervous system. This suggestion is directly supported by the observations of Feldman , who noted that chronically adrenalectomized cortisol-supported cats showed defects in synaptic transmission in the brain stem as compared to intact animals. This conclusion is based on his observations that in the multisynaptic structures of the midbrain reticular formation and the ventromedian hypothalamus of the adrenalectomized cats (1) the latency of the evoked potentials

upon sciatic nerve stimulation was prolonged, and indication of slowing in central nervous system conduction; (2) normal recovery was delayed; and (3) susceptibility to pentobarbital was greater as compared with the intact cats. In contrast, adrenalectomy did not change these parameters in the oligosynaptic pathways of the medial lemniscus and the posterolateral hypothalamus.

With respect to the neuromuscular junction, Torda and Wolff demonstrated that adrenalectomy and/or hypophysectomy in rats caused a decline of the amplitude and the area of the muscle action potential during repetitive, indirect stimulation (sciatic nerve). Since the magnitude of muscle contraction during direct stimulation decreased less than during indirect stimulation, it was concluded that the neuromuscular fatigue which occurs in such animals is caused by moderate dysfunction of muscle and marked dysfunction of nerve; the possible role of the myoneural junction was not elucidated. ACTH, and to a lesser extent cortisone, restored the neuromuscular system of the hypophysectomized rats to normal. It is also of interest that Hoppe and Vogel and Vogel and Westphal demonstrated a decreased chronaxie (increased excitability) in the gastrocnemius muscle of adrenalectomized frogs; potassium chloride further decreased the chronaxie lowered by adrenalectomy. Administration of sodium chloride solution or ACE restored muscle excitability to normal. It was postulated that changes in muscle excitability were correlated with changes in the ratio of Na to K concentration in muscle and plasma an observation consistent with the relation which exists between alterations in brain excitability and electrolytes in brain and plasma (see below).

The observations cited do not indicate clearly whether the neuromuscular defect noted in the adrenalectomized animal is located at the myoneural junction or in the nerve itself. Several investigators have studied the effects of adrenalectomy on the function of peripheral nerve and have shown changes in excitability. Hoagland and co-workers

that the excitability of rat sciatic nerve *in situ* was decreased moderately in the adrenalectomized animal and partially, but significantly, restored toward normal by the injection of ACE. However, sciatic nerves removed from normal and adrenalectomized rats and studied in a chamber exhibited no group differences in excitability. It was concluded therefore, that an intact circulation is necessary for the occurrence of a decrease in nerve excitability in adrenalectomized rats.

The effects of adrenalectomy and of various adrenal hormones on the chronaxie of peripheral nerves have been extensively studied by

Chauchard , Lecoq), and Lecoq *et al.* . The chronaxies of the nerves to the extensor and the flexor muscles of the big toe were determined in several species of animals, and of the median and radial nerves in man. Adrenalectomy increased peripheral motor nerve chronaxie (decreased excitability), an observation in agreement with that of Hoagland and co-workers discussed above. The decreased excitability of peripheral nerve produced by adrenalectomy is in sharp contrast to the increased brain excitability which occurs in such animals (see below). However, this apparent discrepancy was resolved by Wright and Lester), who also found, in confirmation of the previous investigators, that adrenalectomy decreased excitability of peripheral nerves obtained from rats and measured *in vitro*. Cortisone treatment prevented the change in excitability induced by adrenalectomy. Wright and Lester showed that the excitability change of nerves from adrenalectomized rats could be duplicated by placing nerves from intact rats in a low Na medium. According to Wright and Lester, therefore, the change in excitability in the adrenal-insufficient animals is a result of the low plasma Na concentration which occurs in such animals. They also pointed out that the effect of adrenalectomy to decrease excitability or increase chronaxie of nerve might be expected to enhance its tendency to respond repetitively. A nerve fiber capable of repetitive firing is thus more excitable than one not possessing this capacity. The effect of adrenalectomy, therefore, is to increase excitability rather than decrease it according to the classical definition of excitability employing the excitation constant or chronaxie. The contradiction between results obtained from brain tissue and nerve tissue is thus resolved.

Summary: It appears from the available evidence that animals with adrenocortical insufficiency exhibit the following changes: decreased central nervous system conduction time, decreased ability to sustain reflex spinal cord activity, decreased excitability of peripheral nerve, and increased excitability of skeletal muscle. The relation of these changes to metabolic alterations in adrenalectomized animals is discussed subsequently.

2. *Hyperfunction*

The influence of the various *adrenocortical hormones* on peripheral nerve have been extensively investigated by Chauchard ( ), Lecoq , and Lecoq *et al.* . These investigators used rats, pigeons, and guinea pigs as experimental animals and also performed some experiments on man. The changes in peripheral nerve excitability (as measured by chronaxie changes) produced by adrenocortical hormones

were as follows: (1) single small doses of deoxycorticosterone (DOC) *increased excitability, whereas large doses decreased excitability; intermediate amounts elicited a diphasic response, hyperexcitability* followed by depression; chronic treatment *with DOC* increased excitability. (2) ACE had an action similar to that of DOC. (3) Cortisone always decreased nerve excitability, whether the dosage was acute or chronic. (4) Corticosterone resembled DOC in its effects on peripheral nerve. (5) Cortisol and 11-deoxycortisol (substance S) had the same effects as cortisone. In addition, Chauchard, Lecoq, and co-workers noted that all the adrenocortical hormones referred to above increased excitability in both preganglionic and postganglionic sympathetic fibers. ACTH decreased excitability of somatic motor nerves. The attempt made by these investigators to explain the changes in peripheral nerve excitability on the basis of changes in acid–base balance produced by the various hormones is quite unconvincing; such an explanation is not compatible with the findings of Hendley *et al.* and Woodbury and Karler that acidosis decreases whereas alkalosis increases nervous system excitability and those of Lorente de Nó that nerve is relatively unresponsive to changes in pH between the limits of 5.5 and 8.0. Furthermore, both DOC and cortisone, in experimental animals and man, produce a hypokalemic, hypochloremic alkalosis and hence have the same effects on acid–base balance despite opposite influences on peripheral nerve excitability. However, the described effects of adrenal steroids on peripheral nerve excitability are opposite to those on brain excitability (see below), and an explanation for this difference is not available at present.

The effects of *adrenocortical steroids* on *reflex activity*, as measured by the righting reflex and other tests, have been investigated by Negrete and del Pozo who noted that large (anesthetic) doses of DOC in rats produced marked depression of reflex activity. When cortisone was given prior to DOC, the depression was shorter in duration, the induction time was longer, and recovery occurred sooner. Thus cortisone antagonizes the effects of DOC on reflex activity as it does on brain excitability. A detailed study of the effects of adrenocortical steroids on spinal activity has not been undertaken.

A fair amount of information is available concerning the influence of *adrenocortical steroids* and *ACTH* on neuromuscular transmission and skeletal muscle function. The effect of ACTH on neuromuscular function of patients with myasthenia gravis was studied by Torda and Wolff who found that the hormone largely prevented the characteristic decline in the amplitude of the muscle action potential

caused by repetitive indirect stimulation. ACTH also improved the work performance of myasthenic patients. In normal rats, Torda and Wolff observed that ACTH and cortisone did not affect the amplitude of the muscle action potentials evoked by indirect or direct stimulation; but in hypophysectomized rats, both agents restored toward normal the decreased amplitude of such action potentials. Thus the hormones improve neuromuscular function only when such function is abnormal.

It is not the purpose of this chapter to discuss in detail the effects of *adrenocortical steroids* on skeletal muscle function. The interested reader is referred to the work of Ingle and collaborators (see Ingle, Ingle and Kuizenga, ; Ingle *et al.*, . However, a brief account of some changes in skeletal muscle function similar to those in the nervous system might prove useful in elucidating the mechanism by which the adrenocortical steroids affect the properties of excitable tissues in general.

It was quite early recognized by Hartman and Thorn that ACE improved the muscular weakness in patients with Addison's disease, and consequently the effect of ACE on various non-Addisonian patients with asthenia was determined. Ergographic measurements in patients with asthenia secondary to Graves' disease, muscular dystrophy, osteomyelitis, and diphtheria showed that ACE increased their ability to work without fatigue; however, patients with neurasthenia and myasthenia gravis were not improved. The marked impairment in muscle strength in acute adrenocortical insufficiency was found to be counteracted by adrenocortical steroids; in order of decreasing potency were ACE, cortisone and cortisol, corticosterone, dehydrocorticosterone, and DOC. However, none of these steroid preparations elevated muscle work output to normal in the adrenalectomized rat, or increased the work capacity in normal animals (Ingle and Kuizenga, ; Ingle *et al.*,

In contrast, del Pozo *et al.* found that, in striated muscle of normal cats, intravenous or intra-arterial injection of cortisone hemisuccinate (water soluble) caused an immediate although rather small and transitory increase in amplitude of the response to indirect electrical stimulation. The enhancement of muscular contraction seemed to result from an action of cortisone on contractile tissue since the same effect was observed in denervated muscle stimulated directly. In addition, these investigators found that cortisone delayed the appearance of the fourth stage (fatigue) of neuromuscular transmission. Given at a time when the fifth stage (synaptic recovery) was present, the steroid pro-

duced an immediate and long-lasting increase in tension, but when given beforehand this state did not appear; the mechanism of these effects of cortisone is not known. Finally, del Pozo *et al.* demonstrated that cortisone increased the resistance of muscle to the effects of ischemia secondary to arterial occlusion.

However, not all clinical data support the view that cortisone improves neuromuscular function. Merritt has summarized the literature on the effects of ACTH and cortisone therapy in 36 patients with myasthenia gravis. In a small percentage of the cases, no clinical improvement was noted; in the majority of patients, the therapy caused a temporary exacerbation of the symptoms, followed by a temporary remission of variable degree. Such remission was rarely complete, and neostigmine was still required. In some cases treated with cortisone, the temporary improvement appeared following withdrawal of the drug during the period of relative adrenocortical insufficiency caused by such therapy. It is of interest in this latter connection that the symptoms of myasthenia gravis may be somewhat ameliorated by the oral administration of large amounts of potassium salts (Goodman and Gilman, 1955), and it is therefore possible that the symptomatic improvement following withdrawal of ACTH or cortisone is the result of an increase in plasma K associated with the relative adrenocortical insufficiency.

Clinical evidence for a direct effect of adrenocortical steroids on muscle itself has been presented by Shy *et al.* who observed that cortisone significantly reduced the myotonic response in patients with myotonia dystrophica; Reese and Peters also noted a decrease in myotonic symptoms in seven patients. Glaser and Merritt however, found no change in the myotonic response in three cases of this disease treated with ACTH or cortisone. Since it has been demonstrated that potassium salts may intensify the symptoms of myotonia congenita (W. R. Russell and Stedman, and that DOC (which lowers plasma and muscle K concentration) may abolish the myotonic response in goats and in man with congenital myotonia (Brown and Harvey, ; Nissen,), it would appear that ACTH and cortisone decrease myotonia by an effect on K metabolism similar to that produced by DOC. Further experimentation is necessary in order to establish the role of adrenocortical steroids in neuromuscular transmission and on the excitatory and contractile processes in muscle. It is of interest, however, that Glaser and Stark made rabbits myopathic by chronic administration of cortisone and found that excitability of muscle in such animals was the same as the controls.

B. Effects on Brain Excitability

1. Hypofunction

The effects of *adrenocortical insufficiency* on brain excitability have been studied by four different techniques: (1) excitability as measured by threshold for electrically induced seizures (EST); (2) excitability as measured by threshold for pentylenetetrazol (Metrazol), strychnine, or insulin-induced seizures; (3) excitability as measured by susceptibility to audiogenic seizures; (4) occurrence of spontaneous seizures.

The effects of *adrenalectomy* on *brain excitability as measured by EST* were studied by Davenport ( ) and Timiras *et al.*) and summarized by Woodbury). Davenport demonstrated that the EST of adrenalectomized rats placed on water to drink decreased progressively (increased excitability) and after 4 days reached a minimum of 23% below the preoperative control threshold; the EST of rats maintained on 0.9% sodium chloride solution, deoxycorticosterone (hereinafter called DOC, regardless of the particular salt), or ACE was the same as that of nonoperated controls. Administration of potassium chloride or magnesium chloride solutions accentuated the decrease in threshold produced by adrenalectomy alone; calcium chloride solution maintained the threshold approximately at normal. These results in rats have been extended to mice by Timiras *et al.* , who found that adrenalectomized mice given water to drink showed a progressive decrease in EST, to both 60-cycle alternating current (25% decrease) and low frequency unidirectional current (25% decrease); adrenalectomized mice given 0.9% sodium chloride to drink exhibited no change in threshold as compared to intact animals. The changes in brain excitability noted by these investigators were found to be correlated with changes in brain and plasma electrolyte concentrations, as discussed later.

The effect of *castration* and *ovariectomy* on EST and maximal electroshock seizure (MES) pattern have been studied by Woolley and Timiras). The EST was slightly decreased in rats castrated *before puberty* but did not change in rats castrated *after puberty.* In *postpuberally* castrated rats, however, brain excitability decreased as shown by changes in the MES pattern: the duration of tonic flexion increased whereas the duration of tonic extension decreased. The ratio of tonic flexion to extension is a standard index of seizure intensity; the greater the ratio the less severe is the convulsion. In contrast to postpuberally castrated rats, prepuberally castrated rats did not show any changes in the MES pattern.

The effects of *ovariectomy* before and after puberty, as reported by Woolley and Timiras , are as follows. Rats ovariectomized *before* puberty demonstrated a decrease in brain excitability as shown by an increase in EST and in flexion to extension ratio. In rats *ovariectomized after puberty* brain excitability did not change even 3 weeks after ovariectomy was performed.

The influence of *adrenocortical hypofunction* on susceptibility to *Metrazol-induced seizures* was measured by Torda and Wolff in adrenalectomized rats maintained on sodium chloride; no change from control values was observed. Cicardo also noted that the dose of Metrazol necessary to produce minimal convulsions was the same in adrenalectomized rats and adrenalectomized and hypophysectomized toads as in the intact controls. Adrenocortical hypofunction, therefore, does not appear to increase susceptibility to Metrazol-induced convulsions as it does to seizures produced electrically. However, the problem of chemically induced convulsions in adrenalectomized animals is complicated by alterations in the absorption, fate, and excretion of the convulsant drug, and it is likely that adrenalectomy influences one or more of these factors; for example, if absorption of the convulsant is delayed, a larger dose would be required to produce a given effect. Although the experiments of Torda and Wolff rule out this particular factor of absorption since the Metrazol was administered intravenously, other factors such as those discussed by Swinyard *et al.* are involved even when the convulsant is injected intravenously. More precise studies are necessary for a complete solution of the problem.

susceptibility to audiogenic seizures when maintained on water but exhibited a decreased susceptibility to audiogenic seizures when maintained on large doses of sodium chloride. The explanation of these findings lies in the protection afforded by sodium chloride against the increase in brain excitability resulting from adrenalectomy. As discussed above, Davenport demonstrated that sodium chloride restored the decreased EST of adrenalectomized rats to normal, and Woodbury *et al.* have shown that excessive quantities of sodium chloride will elevate the EST to levels above normal in intact animals and (to a greater extent) in adrenalectomized animals. Griffiths also showed that adrenalectomy, whether in wild or domestic rats and whether the rats were treated or not, decreased the spontaneous activity of the animals, probably as a result of the muscle weakness so characteristic of adrenocortical insufficiency.

Although there is little clear-cut evidence showing sex differences in susceptibility to audiogenic seizures, a review by Bevan and Chinn indicates that in rats and mice the trend is for males to be slightly more susceptible to audiogenic seizures than females. Werboff and Corcoran have also reported effects of sex hormones on audiogenic seizures. They showed that *castration* had little effect on seizure responses whereas *ovariectomy* resulted in a decrease in seizure response. *Testosterone* administration resulted in a decrease whereas *estradiol*-17β and *progesterone* administration, both in castrated and in ovariectomized rats, resulted in an increased seizure response.

It has been observed, almost from the time that Addison's disease was first recognized, that patients in crises due to adrenocortical insufficiency exhibit convulsive episodes (Cleghorn, ; G. L. Engel and Margolin, . Also, in experimental animals, H. Peiper (see Bruning, 1921) noted that adrenalectomy resulted in convulsions. Swingle *et al.*) were able to evoke convulsions in adrenalectomized dogs by giving them large amounts of water. It was observed that the adrenalectomized dogs convulsed after smaller quantities of water than were required in intact dogs. The normal dogs spontaneously recovered from water intoxication if fluid administration was discontinued at the time of onset of convulsions, whereas otherwise healthy and vigorous adrenalectomized dogs subjected to water intoxication did not recover unless injected intravenously with ACE or hypertonic sodium chloride solution.

Knobil *et al.* studied the effects of adrenocortical insufficiency in rhesus monkeys and noted extreme weakness, occasional convulsions, prostration, and coma; such crises invariably occurred after a period of fasting occasioned by removal of food or by anorexia. The crises were

associated with a marked decrease in blood sugar concentration; administration of glucose resulted in complete recovery from the crises in 5–60 minutes. The conclusion was reached that hypoglycemia was the immediate cause of death (and presumably of convulsions) in the monkeys with adrenocortical insufficiency.

Although most investigators have explained the convulsions on the basis of the hypoglycemia which occurs in adrenal crises, Arnett *et al.* observed that the frequency of cerebral symptoms in adrenodemedullated and adrenalectomized rats given insulin was unrelated to the blood sugar level. In addition, they found that insulin produced cerebral symptoms—depressed reflexes, coma, convulsions, and delta potentials in the electroencephalogram (EEG)—in 84% of adrenalectomized rats but only in 16% of adrenodemedullated rats, although both groups had a similar degree of hypoglycemia. Thus the adrenalectomized animal is more sensitive than the adrenodemedullated animal to insulin-induced coma and convulsions, and it is evident that so-called hypoglycemic convulsions may be due to some other factor(s). The possible factors involved are discussed later in this chapter.

That convulsions are fairly common in Addison's disease has been pointed out by Storrie , who also reported on EEG changes and seizures in four patients with this disease; all four had abnormal EEG's (generally diffuse slow waves), three of the four had grand mal seizures, and all four exhibited cerebral symptoms (confusion, stupor, and paranoid ideas). The fasting blood sugar in these patients was normal, again indicating that the seizures were not hypoglycemic in origin. Therapy with cortisone and DOC improved their conditions and controlled the seizures.

Summary: It has been demonstrated that adrenalectomized animals are more susceptible to seizures, whether induced electrically or audiogenically or by insulin, strychnine, or water intoxication, but such animals apparently are not more sensitive to Metrazol-induced convulsions. Experimental animals and humans with adrenocortical insufficiency exhibit spontaneous seizures during crises. Castration and ovariectomy appear to have much less effect on brain excitability as measured by various indices. In general both conditions tend to make the animals less susceptible to seizures. The relation of such changes in brain excitability to metabolic processes is discussed below

2. *Hyperfunction*

a. Effect on brain excitability as measured by electroshock seizure threshold. (1) *Adrenocorticosteroids.* The effect of DOC on seizure threshold was first tested in rats by Spiegel and Spiegel and Wycis

(1945), who found that this steroid elevated threshold only at high dose levels: related steroids had only a slight effect.

The effects of chronic administration of adrenocortical steroids and ACTH on brain excitability, as measured by changes in electroshock seizure threshold (EST) in rats, have been summarized by Woodbury (1954), who found that DOC increased EST (decreased brain excitability) to the greatest extent, 11-deoxycortisol increased EST slightly, corticosterone had no effect in the dose used (2 mg per rat), 11-dehydrocorticosterone slightly decreased EST (increased brain excitability), and cortisol and cortisone markedly decreased EST (Woodbury, 1952a); ACTH slightly increased EST, an observation confirmed by De Salva *et al.* (1954) and Ercoli and De Salva (1956). The latter workers also noted that rats became tolerant to the effect of chronic ACTH administration. Other steroids, such as cholesterol, pregnenolone, and acetoxypregnenolone, did not affect brain excitability in rats (Woodbury, 1954). However, some of the newer synthetic steroids also influence EST (Mansor *et al.*, 1956). Certain quantitative data of interest are provided by the results of Holtkamp *et al.* (1952), who found, in adrenalectomized rats, a linear relation between the EST effects of cortisone and DOC and the log of the dose.

The influence of various steroids in combination on brain excitability was also tested (Woodbury, 1952a). The DOC-elevated seizure threshold could be lowered by ACTH, ACE, cortisone, cortisol, corticosterone, and dehydrocorticosterone; 11-deoxycortisol, cholesterol, pregnenolone, acetoxypregnenolone, and testosterone did not alter the DOC-elevated threshold. On chronic administration, ACTH and cortisone prevented the DOC-induced elevation in seizure threshold; conversely, ACTH and dehydrocorticosterone partially prevented, and DOC completely prevented, the cortisone-induced decrease in threshold. On acute administration to intact and adrenalectomized rats, corticosterone prevented both the increase in threshold caused by DOC and the decrease in threshold caused by cortisol. The respective effects of DOC, cortisol, and cortisone on threshold were greater in adrenalectomized than in intact rats (Woodbury, 1954; Woodbury *et al.*, 1951). Administration of 0.9% sodium chloride solution as drinking water enhanced the EST-elevating effect of DOC in both intact and adrenalectomized rats (Woodbury *et al.*, 1951). The interpretation of the above results with steroid combinations will be discussed in Section IV.

The observations in rats, that DOC decreases and cortisone increases susceptibility to seizures evoked by 60-cycle alternating current have been confirmed in mice by Timiras *et al.* (1954) by both the 60-cycle alternating current and low frequency direct current threshold methods.

The changes in electrolytes found by these workers to accompany the changes in brain excitability in mice are discussed below (Section III, A, 1).

(2) *Sex steroids*. Early observations by Spiegel and Wycis (1945) showed that DOC, testosterone, and progesterone had slight anticonvulsant effects as measured by an elevation of EST. More extensive studies, however, of the effects of sex steroids on brain excitability as measured by the EST and MES techniques have been performed by Woolley *et al.* (1960) and Woolley and Timiras (1962a, b). The results are summarized as follows. In rats castrated *postpuberally* testosterone did not alter the threshold as compared with those of intact, untreated controls. Daily administration of high doses of *testosterone* to rats castrated *before puberty* increased the electroshock seizure threshold, whereas low and moderate doses had no effects.

Administration of *estradiol*-17β to intact mature males and to ovariectomized immature and mature females markedly lowered seizure thresholds. The EST-lowering effect of estradiol was directly proportional to the dose. The minimal effective dose of estradiol on the electroshock threshold lies between 0.25 and 4.0 μg/100 gm body weight; this dose lies in the physiological range. Progesterone rapidly and significantly raised the seizure threshold in female rats but had no immediate effect in males. This anticonvulsant effect of progesterone in females persisted for 10 days. After that time the EST was slightly less than that of the intact female controls. *Testosterone* and *methylandrostenediol* (17α-methylandrost-5-ene-3β,17β-diol) significantly lowered seizure threshold in intact adult male rats; this fall in threshold was less marked than that produced by estradiol-17β.

It can be concluded that physiological levels of ovarian hormones influence brain excitability in the rat as measured by EST and MES techniques. Mature female rats exhibited a more severe seizure than did *prepuberally* ovariectomized rats. Since estrogens are the principal ovarian hormones in the mature cycling rat (Emmens, 1959; Segal, 1958), it can be concluded that estrogens exert an excitatory action in the central nervous system. That this excitatory effect of estradiol is a direct one, and not mediated via stimulation of adenohypophyseal tropic hormones, is demonstrated by the fact that this steroid lowers EST even in hypophysectomized rats (Woolley and Timiras, 1962a).

Further evidence for the excitatory effect of estrogens is shown by the changes in threshold and pattern of electroshock seizures during the estrous cycle of the rat (Woolley and Timiras, 1962c). It was observed (a) that the threshold for minimal seizures was highest during diestrus, lower during proestrus, and lowest during estrus; and (b) that the

duration of the tonic flexor component was longest during diestrus and shortest during estrus. The increased brain excitability during estrus is probably due to the high levels of circulating estradiol during this phase.

Summary: It is evident that the adrenocortical steroids produce definite changes in brain excitability, as measured by the EST technique. The steroids that predominantly affect electrolyte metabolism (DOC, 11-deoxycortisol) decrease excitability; those that predominantly affect carbohydrate metabolism (cortisone, cortisol) increase excitability; those with intermediate metabolic effects (corticosterone, 11-dehydrocorticosterone) have intermediate effects on excitability. The relation of the chemical structure of adrenocortical steroids to their action on brain excitability has been described by Woodbury (1952a, 1954). The sex steroids also affect brain excitability as measured by EST. Testosterone and progesterone slightly decrease excitability, whereas estradiol in physiological doses markedly increases excitability. The level of excitability is higher in female than in male rats. Brain excitability also varies with the estrous cycle; excitability is highest in estrus and lowest in diestrus. The relation of the brain excitability changes to the influence of adrenocortical and sex steroids on brain electrolyte, carbohydrate, and protein metabolism is discussed below (Section III).

b. Effect on brain excitability as measured by central chronaxie and by direct stimulation of cerebral cortex. The effects of *adrenocortical hormones* on central excitability as measured by chronaxie in animals have been studied by Chauchard (1952), Lecoq (1954), and Lecoq *et al.* (1952, 1955). Their results were as follows: DOC and ACE increased central excitability; cortisone, cortisol, and 11-deoxy-17-hydroxycorticosterone decreased central excitability; corticosterone increased excitability only after chronic administration. These results are opposite to those reported by investigators using other techniques. Even clinical reports indicate that DOC-like and cortisone-like steroids have effects opposite to those described by the French investigators. It appears likely that the measurement of central chronaxie is not an accurate method for measurement of brain excitability.

The effects of *testosterone* propionate, *estradiol* benzoate, and *progesterone* on central excitability as measured by chronaxie in male and female rats have been reported by Chauchard (1946–1947). All three hormones were reported to increase brain excitability in both male and female animals. Again, it appears that results obtained by this method differ from those obtained by more sensitive and accurate techniques.

In a series of articles, Pasolini (1951, 1952) reported the effects of *adrenocortical steroids* and *ACTH* on seizures evoked by electrical

stimulation of the cerebral cortex in dogs; the study was undertaken because of the observation of Longo cited by Pasolini (1951) that normal dogs became predisposed to evoked seizures after they were adrenalectomized. In intact and adrenalectomized dogs, ijnection of DOC increased the threshold for direct stimulation of the cerebral cortex and prevented seizures in some animals. In sharp contrast, cortisone and ACTH decreased the cortical threshold and increased the incidence of evoked convulsions, and cortisone antagonized the effects of DOC. The above-described effects of the adrenocortical steroids on brain excitability as measured by direct cortical stimulation in dogs are in agreement with those obtained by the EST technique in rats and mice, except that ACTH increases brain excitability in dogs but produces little or no change in rats. This single discrepancy can be explained by the fact that ACTH in rats increases the adrenal output of corticosterone, a steroid which does not alter brain excitability, whereas ACTH in dogs enhances adrenal secretion of cortisol, a steroid which increases brain excitability.

The phenomenon of cortical "irradiation" (spread of the response from electrically stimulated areas of cerebral cortex to distant areas) was quantified in rabbits by Misrahy and Toman (1953), and the effect of cortisone was determined. Ipsilateral electrical responses were compared with concurrent contralateral responses, and thresholds were measured on both sides for primary fast response, secondary slow wave responses, and secondary spindles. Cortisone reduced the ratio of contralateral to ipsilateral threshold for all responses (an excitant effect of cortisone), despite variable effects on ipsilateral thresholds. Such results emphasize the belief that factors other than a direct change in neuronal threshold may predominate in determining the overall "excitant" or "depressant" action of pharmacological agents.

c. Effects on chemically induced seizures in animals. The effects of *ACTH* and *adrenocortical steroids* on the sensitivity of rats to pentylenetetrazol (Metrazol) seizures have been studied by several investigators (Leonard *et al.*, 1953; Swinyard *et al.*, 1955; Torda and Wolff, 1951, 1952a). Acute administration of ACTH and cortisone increased susceptibility to Metrazol (Torda and Wolff, 1952a); in sharp contrast, chronic administration of ACTH decreased the susceptibility to such seizures (Torda and Wolff, 1952a). Leonard *et al.* (1953) found that neither cortisone nor DOC influenced the susceptibility of mice to Metrazol. However, Swinyard *et al.* (1935) observed that DOC increases susceptibility of mice to intravenously injected Metrazol, an effect opposite to its influence on electroshock seizures. DOC was found to protect against seizures induced by cocaine in dogs (Aird, 1944).

Methionine sulfoximine has been shown to be the convulsant substance in agene-treated flour (Mellanby, 1946; Reiner *et al.*, 1950). Various *steroid* hormones have been examined for their influence on seizures induced in dogs by injection of agenized zein. DOC and progesterone protected against "agene"-induced seizures, whereas ACTH and cortisone exacerbated such seizures as evidenced by a decrease in their time of onset and an increase in their severity (Costa and Bonnycastle, 1952). Thus, as is true for electrically induced seizures, DOC decreases and cortisone increases brain excitability. The increase in seizure susceptibility induced by ACTH in agene-treated dogs can be explained by the fact that ACTH in this species causes the secretion mainly of cortisol; this steroid increases brain excitability.

The effects of large (anesthetic?) doses of steroids on chemically induced seizures in animals appear to be different from smaller doses of such steroids. For example, prednisolone, which in smaller doses exerts an excitatory effect on the nervous system (Mansor *et al.*, 1956), is a depressant in large doses (100 mg/kg) and raises the convulsive threshold for intravenous strychnine in mice; however, the convulsive threshold for intravenous Metrazol was not altered (Bonta and Hohensee, 1960).

The effects of *sex steroids* on chemically induced seizures have not been investigated directly. However, Clark and Sarkaria (1958) found that female mice were much more susceptible to acid fuchsin-induced seizures than were male mice. This difference in convulsive susceptibility is probably due to the central excitatory effects of estradiol.

d. Effects on audiogenic seizures. A relation between the *adrenal cortex* and the susceptibility to audiogenic seizures has been postulated by a number of investigators (Colfer, 1947; Ginsberg and Roberts, 1951; Hurder and Sanders, 1953; Vicari *et al.*, 1952). However, the experimental results provide no clear-cut picture of the effects of various adrenal steroids and ACTH on sound-induced seizures. Colfer (1947) found that DOC decreased susceptibility of rats to seizures induced by an air blast; he also noted that brain electrolytes changed as a result of DOC treatment. In 30-day-old dba mice with an allegedly predictable seizure incidence, cortisone raised the incidence of convulsions in females to that characteristic of the males of this strain (Ginsberg and Roberts (1951)). On the other hand, Vicari *et al.* (1952) observed that pregnenolone moderately decreased, and cortisone and ACE slightly decreased, not only the incidence of audiogenic seizures in mice but also the resulting high mortality. Hurder and Sanders (1953) found that ACTH did not influence the susceptibility of rats to audiogenic seizures; the adrenals of their seizure-susceptible group were larger than those of

the control nonsusceptible group; whether the large adrenals were the cause or the result of the seizures was not determined.

The effects of *sex steroids* on audiogenic seizures have not been adequately investigated in intact animals. However, their effects in gonadectomized rats are described above (see Section II, B, 1).

e. Effects of steroids on seizure incidence in experimental animals and man. Further evidence for a central excitatory effect of the *adrenocortical steroids* of the cortisone type is provided by the fact that convulsive episodes occasionally occur in experimental animals and in man treated with such steroids. In rabbits, Pincus *et al.* (1951) noted that ACTH treatment often produced convulsions, particularly when the dose was high. When newborn mice and rats were treated for 3 days with cortisone, Hicks (1953) reported that convulsive episodes developed in the second or third week; pathological changes were also observed in the brains of these animals. Adult animals similarly treated did not exhibit seizures. These results demonstrate the extreme susceptibility of young animals to factors favoring convulsions. The effects of intrathecal administration of soluble cortisol succinate and soluble prednisone-21-phosphate on the CNS of dogs were studied by Oppelt and Rall (1961). They observed that within 5–30 minutes after injection of these steroids into the cisterna magna severe tonic-clonic seizures were produced; such seizures lasted for 15 minutes or longer and often required sodium pentobarbital for control. The convulsive effect of both steroids was dose dependent, and only small amounts were required. Insoluble cortisol suspension in saline had no CNS excitatory effects on intrathecal injection. Thus the cortisol-like steroids have marked direct excitatory effects when placed in the cerebrospinal fluid (CSF), where they reach the CNS rapidly and in high concentrations.

Many clinical reports attest to the fact that both cortisone and ACTH increase brain excitability in man. In patients with no previous history of seizures, generalized convulsions have occurred during treatment of collagen diseases with ACTH and cortisone (Astwood *et al.*, 1950a,b; Baehr and Soffer, 1950; Bonham, 1953; Dameshek *et al.*, 1950; Elkinton *et al.*, 1949; Geppert *et al.*, 1952; Irons *et al.*, 1951; Lowell *et al.*, 1951; Pine *et al.*, 1951; Wayne, 1954). Status epilepticus has also resulted (Dorfman *et al.*, 1951; Stephen and Noad, 1951). In patients with lupus erythematosus, a disease known to affect the central nervous system and to cause convulsions, therapy with cortisone and ACTH has resulted in variable effects, including precipitation of seizures (Baehr and Soffer, 1950) as well as a decrease in seizure frequency (P. W. Russell *et al.*, 1951). Since ACTH elicits the secretion mainly of cortisol in man, a steroid which increases brain excitability, and mainly

of corticosterone in rats (see Woodbury, 1954), a steroid which has little influence on brain excitability, it is evident why ACTH predisposes to seizures in man but not in rats.

Although spontaneous seizures in Cushing's disease are said to be infrequent (Glaser, 1953a), Starr (1952) reported that 4% of his patients had convulsions. There are no reports of spontaneous seizures in cases of adrenogenital syndrome associated with adrenocortical hyperplasia; it is possible that the increased amount of androgenic hormone(s) secreted by the adrenals in this disease protects against any increase in brain excitability which would occur from an excessive secretion of cortisone-like steroids. This possibility is supported by the observation of Henneman (1954) that patients with this syndrome are notably resistant to the excitatory effects of cortisone even when large doses are given.

As early as 1885 Gowers suspected a relationship between epilepsy and *menstruation*. Since then, several investigators have confirmed this observation and agreed that an exacerbation of epileptic seizures occurs just before or at the time of menstruation (Turner, 1907; Almquist, 1955; Ansell and Clarke, 1956). Progesterone recently has been implicated as a factor governing the occurrence of convulsions during the menstrual cycle. Laidlaw (1956) demonstrated a consistent reduction of seizures in the midluteal period of the cycle and an exacerbation of seizures just prior to menstruation He suggested from these results that progesterone exerts an anticonvulsant effect when its levels are high during the midluteal phase but as the levels fall abruptly just before menstruation, withdrawal hyperexcitability occurs which leads to an increased incidence of seizures. More recent studies by Logothetis *et al.* (1959) confirm the findings of Laidlaw with respect to the increased seizure incidence prior to menstruation, but they suggest a different cause for the increased susceptibility than does Laidlaw. Logothetis *et al.* showed that in 25 patients with catamenial epilepsy the seizures occurred primarily during the immediate premenstrual and menstrual periods.

In addition, Logothetis *et al.* noted that intravenous injection or topical application of Premarin (water-soluble estrogenic conjugates) on the brain of normal rabbits or on the brain of rabbits with an irritable cortical lesion produced by an ethyl chloride spray resulted in activation of the EEG. An activating effect of intravenously administered estrogens was also observed in the EEG's of 11 of 16 epileptic patients. On the basis of these results Logothetis *et al.* suggested that the increased incidence of seizures prior to menstruation is due to increased plasma levels of estrogens, not to the sudden decrease in

levels of progesterone that occurs during this period as suggested by Laidlaw. However, the fact that progesterone decreases brain excitability whereas estrogen increases it as discussed above (Section II, B, 2) suggests that both effects, i.e., increased plasma estrogen levels and decreased progesterone levels, account for the marked increase in CNS excitability which occurs in the premenstrual-menstrual period.

f. Anticonvulsant effects of steroids. The effect of DOC in epilepsy was first examined by I. McQuarrie and associates (see McQuarrie, 1946; McQuarrie *et al.*, 1942) who found that this hormone decreased incidence of spontaneous grand mal seizures in two patients and also antagonized the seizure-evoking effect of hydration produced by vasopressin administration. These provocative observations were not confirmed by Aird (1944), who reported that DOC did not decrease the incidence of grand mal seizures in two epileptic patients. In a later paper, however, Aird and Gordan (1951) demonstrated that DOC, given in combination with conventional anticonvulsant drug therapy to otherwise refractory epileptics, reduced the incidence of petit mal and to some extent of grand mal seizures. The effects of ACTH, DOC, and cortisone in 6 epileptic children, 5 of whom were on anticonvulsant drug therapy, were tested by Klein and Livingston (1950); 4 of the 6 were benefited by ACTH; and one (with pure petit mal) showed no definite improvement. The single patient not on drug therapy was not benefited by DOC, cortisone, or ACTH; in fact, the ACTH had to be withdrawn because of the appearance of choreiform movements, motor deficit, and abnormal personality. Other investigators have not found *cortisone* or *ACTH* to be of any value in convulsive disorders; indeed, they must be considered as contraindicated because of their marked tendency to increase rather than to decrease brain excitability, and to predispose to, or actually precipitate, seizures. Only rarely is DOC employed in epilepsy; in an occasional refractory case, it has been temporarily used as an adjuvant agent. Since 11-deoxycortisol has been shown to elevate EST in rats (Woodbury, without producing significant electrolyte distortions, its usefulness in combination with conventional anticonvulsant therapy in epileptic patients merits investigation.

or normalized the markedly abnormal EEG in many of the subjects. The younger the infant when the ACTH or cortisone treatment was started, the better the response (Millichap and Bickford, 1962). Miribel and Poirier (1961) reported that ACTH and adrenocortical steroids have anticonvulsant effects in patients who have not reached full maturity. These hormones were most effective in children with petit mal but were less effective in children with other types of epilepsy. The mechanism of the seizure-enhancing effect of adrenocortical steroids in adults and their seizure-preventing effects in immature persons is not known. However, the observations of Vernadakis and Woodbury (1960, 1963b) that cortisol accelerates maturation of the nervous system, as measured by the EST and development of the tonic-clonic pattern on maximal electrical stimulation, when given to young rats between the ages of 1 and 4 or 8 and 13 days of age, but delays maturation when given between the ages of 4 and 8 days, is pertinent to the observations described in man. They suggested that cortisol accelerates maturation of the CNS by enhancing myelination. Perhaps in young patients with convulsive disorders, ACTH and adrenocortical steroids enhance myelination and thereby prevent seizures and normalize the abnormal EEG. Further studies along these lines are indicated.

Although *testosterone* and *progesterone* have been found to exert anti-convulsant effects in experimental animals (Spiegel, 1943; Spiegel and Wycis, 1945; Woolley and Timiras, 1962a,b), their effects in epileptic patients have not been adequately investigated.

g. Effects on the responses of animals to centrally acting drugs. (1) *Hypnotics.* It is well known that the *adrenalectomized* animal is more sensitive to various drugs, including those acting on the central nervous system. At least two explanations are possible. The first is that, since the regulatory influence (see below) of adrenocortical hormones on the central nervous system is removed, the brain is more sensitive to drugs that produce either excitation or depression. The second is that the absorption, fate, and excretion of the drugs are modified so that their dose-effect curves are shifted. An examination of the literature provides evidence for both explanations. That the adrenalectomized animal is more sensitive to the central nervous depressants is indicated by the following data. Sindram (1935) observed that adrenalectomized rats were more susceptible to urethan. The toxicity of thiopental was greater in adrenalectomized than in normal rats (Eichholtz *et al.*, 1949; Richards, 1941). Tureman *et al.* (1951, 1952) and Robillard *et al.* (1954) noted that the duration of pentobarbital-induced sleep was approximately doubled in adrenalectomized rats as compared to intact controls; cortisone decreased the susceptibility of both groups to pentobarbital;

DOC did not affect pentobarbital-sleep time of adrenalectomized rats in low doses, but prolonged it slightly in high doses. In addition, adrenalectomy was found by Shibata and Komiya (1953) to induce a four-fold prolongation of sleep induced by thiopental; pretreatment with cortisone or cortisol prevented this prolongation, but DOC and isotonic sodium chloride solution were ineffective. Komiya and Shibata (1956) also observed that adrenalectomy decreased the induction time and prolonged the duration of intravenous barbital anesthesia in mice, and that pretreatment with cortisone or cortisol prevented such changes; DOC and isotonic sodium chloride solution had no effect.

Evidence that adrenalectomy prolongs barbiturate-induced sleep by modification of the absorption, fate, or excretion of the drug is based on the following considerations. Adrenalectomy impairs absorption of drugs from the gastrointestinal tract and from parenteral sites of injection, mainly as a result of the inadequate circulation in the adrenalectomized animals. The prolonged sleep time could possibly be explained by impaired drug absorption if the absorption took place over a more protracted period and at a rate sufficient to maintain sleep. Since this possibility is unlikely, another explanation is necessary. That impaired hepatic detoxification of pentobarbital is the cause of the prolonged sleep time observed in adrenocortical insufficiency is suggested, but not proved, by the experiments of Robillard *et al.* (1954), who demonstrated that pentobarbital was metabolized at a slower rate by the liver of adrenalectomized rats than by the liver of intact rats; cortisone was thought to exert its effect by enhancing hepatic degradation of pentobarbital. Komiya and Shibata (1956) attributed the prolongation of barbital anesthesia by adrenalectomy to a demonstrated higher concentration of barbital in the brain; cortisone and cortisol prevented the increase in brain barbital concentration and shortened the duration of anesthesia, whereas DOC had little effect. Since barbital is not metabolized in the body, these data suggest an influence of adrenocortical steroids on the tissue distribution of the drug; although an effect of these steroids to hasten the renal excretion of barbital has not been ruled out, such a change would probably tend to lower the barbital concentration in the brain. Measurements of the plasma-to-brain ratio of barbital and of the rate of excretion of barbital in the urine are needed to obtain an answer to this problem.

There is no evidence that the enhancement of barbiturate-induced anesthesia by adrenalectomy is due to a central effect of the lack of adrenocortical steroids; however, there is evidence that the influences of certain *adrenocortical steroids* on drug-induced depression are due, at least in part, to their central effects. Cortisone and cortisol increase

brain excitability, whereas DOC decreases it. Thus the decrease produced by cortisone in the duration of pentobarbital-induced sleep in adrenalectomized rats, and the delay produced by cortisol in the onset of barbital anesthesia in adrenalectomized mice are probably explainable on the basis of the central excitatory effects of cortisone and the central depressant effects of DOC.

The effects of adrenocortical hormones on the duration of sleep induced by hexobarbital in mice have been studied by Winter and Flataker (1952), who found that cortisone and ACTH shortened sleeping time whereas DOC had no effect. In addition, the markedly prolonged sleeping time of mice receiving a combination of hexobarbital and diphenhydramine was also decreased by cortisone, but the effect of the steroid could only be fully accounted for by its antagonism to the action of barbiturate; thus cortisone was without influence upon the central depressant effects of diphenhydramine. Winter and Flataker concluded that cortisone was an "analeptic" drug, but that its central stimulant action was different in nature from that exhibited by caffeine and amphetamine.

In contrast to low doses, however, large doses of prednisolone exert a depressant effect on the nervous system as shown by the observations that this steroid prolonged markedly the sleep time in mice given hexobarbital or the anesthetic steroid, 3-hydroxy-11,20-diketopregnone-21-hemisuccinate (Bonta and Hohensee, 1960)

(2) *Anticonvulsants.* The anticonvulsant drug diphenylhydantoin moderately elevates the EST of salt-maintained adrenalectomized rats, whereas it has little or no effect on the EST of intact controls (Woodbury, 1954; Woodbury and Goodman, 1953; Woodbury *et al.*, 1957). The diphenylhydantoin-elevated EST of intact or adrenalectomized rats can be prevented by the administration of cortisone. Thus the central effects of this anticonvulsant can be modified both by an excess and a deficiency of adrenocortical steroids.

The anticonvulsant effects of diphenylhydantoin, phenobarbital, and trimethadione (elevation of EST) can be enhanced by DOC (Woodbury, 1952b). Also diphenylhydantoin and phenobarbital protected against the increase in excitability produced by chronic treatment with cortisone (Fingl *et al.*, 1952). On withdrawal of the anticonvulsant drugs, however, the hyperexcitable state was restored within 2 days; this indicates that these anticonvulsant drugs merely mask, but do not prevent, the increase in excitability induced by cortisone.

(3) *Analgesics.* In a well-controlled series of experiments, Winter and Flataker (1951) studied the effects of DOC, cortisone, and ACTH on the responses of animals to analgesic drugs. The reaction time of the

tail-flick response to thermal stimuli was measured in normal rats under various treatments. Cortisone, DOC, and ACTH did not alter normal reaction time; but cortisone and ACTH markedly reduced and DOC increased the reaction time prolonged by morphine or methadone in intact and spinal rats. In addition, cortisone exerted a synergistic effect with the narcotic antagonist, nalorphine, enhanced the well-known excitatory effect of morphine in cats, and moderately increased the LD_{50} of methadone in mice. Since death from methadone results from respiratory depression, it is likely that cortisone, by increasing central excitability, antagonizes the methadone-induced respiratory depression. Since cortisone inhibited the effect of morphine in spinal animals and antagonized the hypnotic and respiratory depressant effect of the analgesic drugs, Winter and Flataker concluded that cortisone is stimulatory to the entire cerebrospinal axis. This conclusion is certainly in harmony with the evidence so far presented in this chapter. DOC was found by Loewe (1956) to diminish morphine-induced mania in cats, an observation previously made by Winter and Flataker.

Cortisone and ACTH reduced the increased mortality produced by morphine in guinea pigs subjected to decompression to a simulated altitude of 25,000 feet for 6 hours (Sobel *et al.*, 1960). The decreased mortality is probably due to the antagonism by cortisone, which increases excitability, of the depressant effects of both morphine and severe hypoxia.

(4) *Anesthetics*. The anesthetic effects of the steroids are discussed in Chapter 8, Volume III, and in the review by Woodbury (1958), hence will not be considered here. However, their effects on anesthesia induced by anesthetic agents will be discussed briefly. The effects of DOC and cortisone acetate on the threshold for nitrous oxide anesthesia in rats were studied by Rummel (1959) and Rummel and colleagues (1959). They observed that DOC lowered and cortisone raised the nitrous oxide anesthetic threshold. Thus DOC has depressant and cortisone excitatory effects on the nervous system as measured by this test, an observation that confirms the data already presented in preceding sections of this chapter. Neither progesterone nor testosterone influenced the anesthetic threshold for nitrous oxide in such rats.

C. Effects on Recovery Processes in the CNS

In addition to their effects on excitable processes in the central nervous system, the *adrenocortical steroids* also influence central recovery processes. For example, in patients undergoing insulin-shock therapy, cortisone was found to be markedly beneficial in hastening

emergence from deep hypoglycemic coma and decreasing postictal depression (Habelmann, 1952). Experimentally, cortisol shortened the duration of the postictal depression that follows electroshock seizures in animals pretreated with insulin (Timiras *et al.*, 1956); the decrease in blood sugar level produced by insulin in these animals was also prevented by cortisol administration.

Chronic treatment of rats with cortisol was found to shorten the recovery time (RT_{50}) from maximal electroshock seizures and to increase the blood sugar concentration. Chronic treatment with DOC, however, did not modify the RT_{50} or the blood sugar concentration (Timiras *et al.*, 1956). The relation of the changes in recovery time to changes in blood sugar is discussed below (Section III, C). The tonic-clonic pattern of the maximal electroshock seizure was also modified by cortisone (Timiras *et al.*, 1956). The duration of tonic extension was lengthened. The increase in duration of the tonic extensor component is an indication of increased brain excitability, an observation which is in agreement with the previously noted decrease in EST produced by cortisone. The effects of ACTH and of steroid hormones other than DOC, cortisone, and cortisol on the recovery process have not been studied.

The effects of sex steroids on central recovery processes have not yet been adequately studied. Woolley and Timiras (1962a) noted that *castration* lengthens postictal depression; however, *testosterone* administration to such rats did not shorten recovery time to that of the intact controls.

D. Effects on Electrical Activity of the Brain

1. Hypofunction

a. Adrenal cortex. The EEG effects of hypocorticism in man were first reported by G. L. Engel and Margolin (1941, 1942), who found a characteristic picture of diffuse, slow activity with frequencies of 2–6/sec and voltages higher than those of alpha waves (up to 75 μv). Hyperventilation induced EEG changes more easily in patients with Addison's disease than in normal subjects, and glucose administration prevented such alterations. ACE, but not DOC, restored the EEG pattern to normal. A rough correlation was found between the EEG abnormalities and blood sugar level, and the EEG appeared to be improved by factors that improved carbohydrate metabolism, such as adequate replacement therapy in cases of Addison's disease.

The observations of Engel and Margolin in man have been amply

confirmed by many investigators (Bricaire *et al.*, 1953; Condon *et al.*, 1954; Forsham, 1951; Forsham *et al.*, 1949; Hoffman *et al.*, 1942; Storrie, 1953; Thorn *et al.*, 1949). Hoffman *et al.* (1942) observed that 18 of 25 patients with Addison's disease exhibited definite abnormalities in the pattern of their resting EEG's. The changes were characterized by: (1) oscillations (5–8/sec) slower than the normal alpha rhythm, with a predilection for the frontal area and a relative refractoriness to the usual effect of opening the eyes; (2) an unusual exaggeration of the normal EEG response to voluntary hyperventilation; and (3) a reduction in incidence of low-voltage, high-frequency activity (beta waves). The EEG abnormalities progressed despite DOC therapy; in addition, treatment with ACE, vitamin B complex, and a diet high in carbohydrate failed to correct the EEG. Restoration of blood pressure, plasma volume, and electrolyte concentration prevented neither the occurrence of changes in the resting EEG nor the sensitivity to hyperventilation. Gorman and Wortis (1947) also described high-voltage, slow bursts in all leads in the EEG of a patient with Addison's disease; the abnormality was exaggerated by hyperventilation. Thorn *et al.* (1949) and P. H. Forsham *et al.* (1949) reported that cortisone abolished the EEG alterations (essentially the same as those reported by Engel and Margolin and by Hoffman *et al.*) in patients with Addison's disease; however, "nervousness" was not relieved. Corticosterone has been observed to correct both the EEG abnormalities and the nervousness and excitability of patients with Addison's disease and, like cortisone, to produce a feeling of well-being (Conn *et al.*, 1951). 11-Dehydrocorticosterone appears to produce the same corrective results as corticosterone (Forsham, 1951).

It is of interest that the dominant frequency of the EEG is below normal in adrenalectomized rats as well as in patients with Addison's disease; on the other hand, hypophysectomy in rats does not change the EEG (Bergen, 1951; Bergen *et al.*, 1953; Torda and Wolff, 1952a). The normal pattern can be restored by treating adrenalectomized rats with ACE and pregnenolone, but not with DOC (Bergen, 1951; Bergen *et al.*, 1953). The relation between the EEG and metabolic changes is discussed in Section III.

b. Gonads. The effects of gonadal insufficiency or gonadectomy on electrical activity of the brain have not been adequately studied.

2. *Hyperfunction*

a. Effects of steroids on electrical activity in man. EEG changes induced by *adrenocortical steroid* hormones and ACTH have been reported by many investigators (Boland and Headley, 1949; Conn *et al.*,

1951; Debre *et al.*, 1952a,b; Forsham, 1951; Friedlander and Rottger, 1951; Friedman and Engel, 1956; Glaser and Merritt, 1952; Glaser *et al.*, 1955; Gottschalk, 1952; Hoefer and Glaser, 1950; Nekhorocheff, 1952; Pine *et al.*, 1951; Ragan *et al.*, 1949; Ransohoff *et al.*, 1951; Streifler and Feldman, 1953; Ulett *et al.*, 1951; Wayne, 1954; Wayne and Boyle, 1953). The first extensive report of the effects of ACTH and cortisone on the EEG in man was that of Hoefer and Glaser (1950), who found that ACTH and particularly cortisone caused the appearance of a significant amount of moderately slow wave activity (4–7/sec), and an increase in sensitivity to hyperventilation; however, the incidence of these changes was variable. In 4 epileptic patients, Klein and Livingston (1950) observed that ACTH therapy decreased the incidence of seizures and improved the EEG pattern. In contrast, Glaser and Merritt (1952) noted that ACTH and cortisone increased the incidence of abnormalities in the EEG in epileptic patients and did not reduce the frequency of seizures. It is of interest that all but one of the patients treated with ACTH by Klein and Livingston (1950) were on standard anticonvulsant medication; the patient not on an anticonvulsant developed a severe reaction (choreiform movements, and abnormal personality) which necessitated withdrawal of the ACTH. In view of the fact that diphenylhydantoin and phenobarbital protect against cortisone-induced hyperexcitability in rats (Fingl *et al.*, 1952), it seems likely that, in the patients observed by Klein and Livingston, any central excitatory effects of cortisone and ACTH were masked by the concomitant anticonvulsant; in the single case in which anticonvulsant medication was not used, excitatory phenomena appeared.

The effects on the EEG produced by intravenous administration of cortisol and ACTH were measured by Glaser *et al.* (1955), who found that both hormones increased the activity (5–7/sec) in the EEG. Cortisol seemed to exert this effect more consistently and to a greater degree than did ACTH. The effect was somewhat greater in epileptic subjects with previously abnormal EEG's. In two epileptic patients cortisol increased the incidence of 2–3/sec spike-wave seizure discharge. No correlation between EEG alterations and serum electrolyte changes was found, depite the fact that there was an increase in serum K concentration in 5 of the 7 patients given cortisol intravenously.

A tentative hypothesis for the mode of action of cortisone on the EEG has been advanced by Streifler and Feldman (1953), based on the following observations: ACTH and cortisone act on the hypothalamus and thalamus; electrical stimulation of the basal structures of the brain modifies the electrical activity of the cortex in experimental animals; hypothalamic lesions slow cerebral cortical activity. These observations,

coupled with those of Castor *et al.* (1951) that cortisone and ACTH cause pathological changes in the hypothalamus and thalamus, led Streifler and Feldman (1953) to suggest that the EEG changes induced by these hormones may, to some extent, be due to their direct action on diencephalic structures. The fact that most of the EEG changes induced by the 17-oxysteroids are in the direction of slower activity is compatible with the hypothesis of Streifler and Feldman, and indicates more specifically an effect either to depress the mesencephalic reticular activating system or, more likely, to enhance the recruiting response in the diffuse thalmic projection system (Penfield and Jasper, 1954). The fact that cortisone has general excitatory properties on the nervous system indicates that probably it is acting to enhance the recruiting response in the thalamus, rather than to depress the arousal response from the mesencephalic reticular activating system. It could, however, excite inhibitory pathways in this area and thereby produce the observed effects on the EEG. No evidence for or against this possibility is available. The fact that 17-oxysteroids decrease the low-frequency EST (Timiras *et al.*, 1954) suggests that this steroid does enhance the recruiting response in the diffuse thalamic projection system. Low-frequency seizures appear to originate in this area and then spread to the cerebral cortex (D. McQuarrie and Fingl, 1957). Further research to localize the anatomical sites of cortisone action is needed. It is of interest in this connection that Feldman *et al.* (1961) have demonstrated that the glucocorticoids exert marked effects on the brain stem in cats. They measured the effects of glucocorticoids on potentials in the medial lemniscus, midbrain reticular formation, different regions of the hypothalamus, and intralaminar nuclei of the thalamus evoked by stimulation of the contralateral sciatic nerve. Cortisol and ACTH caused an increase in the negative potentials of the multisynaptic brain stem systems (an excitatory effect) shortly after their administration. However, there was almost no change in the positive evoked potential of the lemniscal response. These experiments of Feldman *et al.* demonstrate that administration of adrenocortical steroids increases excitability in brain centrencephalic structures of cats (reticular formation, hypothalamus, etc.) and provide substantial evidence for the hypothesis of Streifler and Feldman (1953) described above that the glucocorticoids exert a direct effect on diencephalic structures. These subcortical effects could account for the manifold effects of cortisol on electrical activity of the brain, seizure activity, consciousness, and behavior; such effects are regulated by multisynaptic reticular systems in the midbrain and diencephalon.

Corticosterone and 11-dehydrocorticosterone have both been shown

to correct the EEG abnormalities in patients with Addison's disease (Conn *et al.*, 1951; Forsham, 1951), but these steroids have not been studied for their EEG effects in patients with collagen diseases or in normal individuals.

DOC has little influence on the EEG; however, Aird and Gordan (1951) did note a small, but consistent, decrease in the frequency of abnormal waves in 6 epileptic patients treated with this steroid. Ward *et al.* (1954) found that aldosterone did not alter the EEG pattern of patients with rheumatoid arthritis.

Dusser de Barrene and Gibbs (1942) studied variations in the electroencephalogram during the *menstrual cycle* and found that sufficient slowing of the EEG occurred in association with menstruation to change a normal EEG to an abnormal one. Gibbs and Reid (1942) demonstrated that in the last weeks of pregnancy (when progesterone levels are high) the electrical activity of the cortex was definitely slow compared to postpartum EEG records. However, the EEG of postmenopausal women was not significantly altered by injections of progesterone or stilbestrol (Cress and Greenblatt, 1945). In contrast to this negative effect of female sex steroids on the EEG of postmenopausal women, in patients with catamenial epilepsy, Logothetis *et al.* (1959) observed an activating effect of water-soluble conjugated estrogens (Premarin) on the EEG in 11 of 16 patients. Thus, further studies are necessary in order to define the effects of female sex steroids on the electrical activity of the brain. The effects of male sex steroids on the EEG have not been described.

b. EEG changes induced by hyperfunctional diseases states: Cushing's disease and androgenital syndrome. EEG changes in *Cushing's syndrome* are similar to those induced by ACTH and cortisone and need be mentioned only briefly (Glaser, 1953c; Plotz *et al.*, 1952; Soffer, 1946). The main change is a decrease in the frequency of the alpha rhythm to a value of 3–7/sec. This shift appears to be correlated with an enhanced sensitivity to seizures. Two of 7 patients in one series developed seizures (Glaser, 1953c). No reports have come to the author's attention concerning the EEG pattern of patients with *adrenogenital syndrome* and other diseases of gonadal hyperfunction.

c. Effects of adrenocortical and sex steroids in experimental animals. Relatively few experiments have been performed to test the effects of *adrenocortical hormones* on the electrical activity of the brain in intact animals, in contrast to animals with adrenocortical insufficiency (Grenell, 1947; Grenell and McCawley, 1947a,b; Ortiz-Galvan and Morrell, 1956; Prados *et al.*, 1945; Soulairac, 1953; Torda and Wolff, 1952a). In cats, ACE has been reported to increase the amplitude and

the frequency of the EEG and to prevent the flattening of waves associated with the edema resulting from exposure of the brain (Grenell, 1947; Grenell and McCawley, 1947a,b; Prados *et al.*, 1945). Similarly, Torda and Wolff (1952a) demonstrated in normal rats that ACTH increased the EEG voltage and produced occasional spiking and paroxysmal runs of low frequency, high-voltage waves. Anoxia-induced disappearance of brain waves in rats was delayed by ACTH administration (Soulairac and Soulairac, 1953). The relation between these reported EEG effects of ACTH and ACE and their effects on brain excitability is not clear from the meager data available.

Experiments on the effects of *sex steroids* on the EEG of experimental animals have been mainly confined to studies on the EEG arousal threshold involving the brain stem reticular formation and the hypothalamus and the role of changes in these parameters in sexual behavior and pituitary function. A discussion of the results of such studies is beyond the scope of this chapter. The interested reader should consult articles by Kawakami and Sawyer (1959) and Sawyer and Everett (1959) for further information.

However, Logothetis *et al.* (1959) noted in rabbits that estrogens exerted a significant epileptogenic effect (activation of the EEG and production of seizures) when applied locally over an irritable cortical lesion produced by ethyl chloride spraying, or on an intact cortex, and when injected intravenously in such animals. Thus the meager data on the effects of sex steroids on electrical activity of the brain of experimental animals indicate an excitatory effect of estrogens on the CNS.

III. Neurochemical Effects of Steroidal Hypofunction and Hyperfunction

A. Effects on Electrolyte and Acid-Base Metabolism

1. *Hypofunction*

a. Adrenal cortex. The effects of adrenocortical hypofunction on brain and plasma electrolyte distribution and brain excitability have been described by Davenport (1949) and Timiras *et al.* (1954) and summarized by Woodbury (1954, 1958). Davenport found that, whereas the plasma Na concentration of adrenalectomized rats given water to drink decreased markedly and plasma K concentration increased markedly, there was no change in total cerebral cortical Na, K, and Cl. However, recalculation of her data indicates that intracellular Na concentration of the brain, predicated on the basis that chloride space is a measure of extracellular fluid volume, was increased and that the ratio of brain extracellular to intracellular Na concentration was decreased.

[It is well recognized that this assumption is incorrect, since chloride is present in high concentrations in glial cells and in low concentrations in neuronal cells. However, it is also true (unpublished observations) that the amount of Na in brain in excess of the amount found in the chloride space is a measure of cellular Na (glial and neuronal) and does vary with the functional state of the brain. Whether the changes are due to glial or neuronal cell Na shifts or both cannot be decided at the present time.] A direct correlation between plasma Na concentration and electroshock seizure threshold was also reported by Davenport.

In adrenalectomized mice given water to drink, Timiras *et al.* (1954) observed a decrease in plasma Na level and an increase in plasma K level, whereas in the cerebral cortex there was a marked increase in total brain Na concentration and no change in K concentration. Calculation of brain intracellular electrolytes revealed a marked increase in Na concentration and no change in K concentration; the ratios of extracellular Na and of intracellular K were decreased. Thus, despite a decrease in extracellular Na, brain Na concentration was increased. In contrast, the concentration of Na in skeletal muscle cells was decreased. The increase in intracellular brain Na (decrease in brain Na ratio) was associated with an increase in brain excitability. Bergen and Hoagland (1951), Stern *et al.* (1951), and Flanagan *et al.* (1950) reported that the total brain Na and K did not change after adrenalectomy in rats and dogs, but brain intracellular values of these cations were not calculated.

Since adrenalectomy increases intracellular brain Na concentration, it is of interest to note the effect of this procedure on the turnover of radiosodium by brain. Stern *et al.* (1951) observed that the relative activity of brain (a measure of turnover rate) of adrenalectomized rats was slightly decreased by adrenalectomy; this finding, coupled with the increase in brain intracellular Na concentration in such animals, indicates that adrenalectomy decreases the active transport of Na out of brain cells (see Section III, A, 2).

The influence of adrenalectomy on radiopotassium turnover by the brain has been studied extensively by Bergen and Hoagland (1951), Bergen *et al.* (1956), Hoagland (1954), Hoagland and Stone (1948) and by Leiderman and Katzman (1953). A summary of their data and of the argument that part of brain K is present as a bound form is found in the review by Woodbury (1958) and the chapter by Hoagland (1962) and will not be discussed here. It appears from the data that the turnover of radiopotassium in brain is probably increased by adrenalectomy, but much more research is required.

In summary, the brain of the adrenalectomized animal exhibits an

increased concentration of intracellular Na, a decreased ratio of extracellular to intracellular Na concentration, and a decreased turnover of Na; total brain K concentration is unchanged, but the ratio of intracellular to extracellular K concentration is decreased and the turnover of K is questionably increased. Accompanying these changes in electrolytes there is an increase in brain excitability, a slowing of the frequency of the EEG, a decrease in central conduction time, and behavioral changes. From the data presented and additional evidence to be discussed later, it is suggested by the authors that changes in brain excitability are correlated with changes in brain Na, whereas some of the other neurophysiological alterations are correlated with changes in brain K and oxidative metabolism.

b. Gonads. Neither castration nor ovariectomy alter the total or intracellular concentrations of Na and K in the cerebral cortex of rats (Woolley *et al.*, 1960; Woolley, 1961) (see Section III, A, 2).

2. Hyperfunction

a. Adrenal cortex. The effects of *adrenocortical steroids* on electrolyte metabolism of tissues other than brain have been examined in considerable detail. DOC has been most extensively studied with respect to tissue electrolytes, including brain, whereas cortisone, hydrocortisone, and ACTH have been less thoroughly examined. In tissue other than brain, it has been shown that DOC causes a rise in intracellular Na concentration and a concomitant loss of intracellular K. In contrast, cortisone and ACTH exert only a mild effect on such tissues. In rats, cortisone causes a slight loss in skeletal muscle Na with little influence on K distribution, whereas ACTH has no effect.

The effects of DOC on brain electrolytes were studied by Woodbury and Davenport (1949), who found that total brain Na and K concentrations were not altered but that brain intracellular Na concentration, calculated on the assumption that chloride space is a measure of extracellular fluid volume (see above), was markedly decreased, although brain intracellular K concentration remained unchanged, the ratio of intracellular to extracellular K in brain was increased by DOC treatment. The decrease in brain intracellular Na was associated with an increase in EST (decrease in brain excitability), an observation consistent with that discussed above for adrenalectomized rats and mice. That a fundamental difference exists in intact rats between the effects of DOC on brain and other tissues (particularly skeletal muscle) is indicated by the fact that only in brain does DOC decrease intracellular Na concentration; in other tissues studied (muscle, heart, liver, skin), intracellular concentration of Na is increased while that of K is decreased

(Woodbury, 1963). This is strong evidence that the decrease in brain excitability induced by DOC is a result of the decrease in brain intracellular Na concentration. The above described brain electrolyte effects of DOC have also been reported by Colfer (1947), Hoagland (1954), and Timiras *et al.* (1954).

The influence of DOC treatment on the turnover of radioactive Na in brain and other tissues of dogs was studied by Overman *et al.* (1951), who found that DOC increased the turnover of brain Na in adrenalectomized, but not in intact, dogs; since the measurement of the Na^{24} uptake was made only 30 minutes after injection of the isotope, it is hard to interpret these results. The authors suggest, on the basis of experiments by one of us (Woodbury, 1963), that correct analysis of the effects of agents on the uptake of radioelectrolytes in brain requires data on the complete activity-time curves for periods up to 24 hours. Although it is likely that DOC actually does increase the turnover of Na^{24} in brain cells, more convincing information is necessary.

Some unpublished experimental results (Timiras and Woodbury, 1957) are of interest in relation to the effects of DOC on brain electrolytes and excitability. DOC administered chronically to unilaterally nephrectomized, castrated, adult rats given 0.9% sodium chloride solution to drink caused a decrease in brain excitability and in brain intracellular Na concentration, an increase in brain extracellular Na concentration, and an increase in the ratio of brain intracellular to extracellular K concentration; cerebral edema did not occur. However, in young rats prepared and treated in exactly the same manner, brain excitability was decreased only during the first 11 days of DOC administration, and then increased until the time of sacrifice for electrolyte analyses on the 50th day; the large (toxic) doses of DOC caused marked cerebral edema and the extracellular water content of the brain was elevated. In these young rats, instead of the usual pattern of electrolyte changes caused by DOC, intracellular brain Na increased despite the fact that the brain K ratio was enhanced. Thus, in nontoxic doses in the adult animals, DOC decreased brain excitability and intracellular brain Na concentration, whereas toxic doses in the young animals increased brain excitability and intracellular Na concentration, a change in Na that DOC produces normally in other tissues. The experimental results indicate that DOC in low doses stimulates the active pumping of Na out of brain cells; but, under special conditions and in the presence of excess Na, this effect on the Na pump fails, toxicity results, and brain excitability increases. The toxic side effects of DOC, such as the convulsions induced in rats by high doses, may be related to this effect on brain Na.

The changes induced by aldosterone in brain and skeletal muscle

electrolytes have been studied in mice (Woodbury and Koch, 1957). This steroid increases the ratio of extracellular to intracellular Na concentration and intracellular to extracellular K concentration in both brain and muscle. Thus aldosterone and DOC produce similar effects on brain electrolytes but opposite effects on muscle electrolytes. This discrepancy was resolved by Withrow and Woodbury (1964), who found that DOC had the same influence as aldosterone on Na and K concentrations in muscle in nephrectomized, partially eviscerated rats. Thus the action of DOC to increase Na and decrease K concentration in muscle of intact animals is secondary to the marked effect of this hormone to increase the renal and gastrointestinal excretion of K. The effect of large doses of DOC to increase intracellular Na concentration in young rats is probably due to the marked increase in renal excretion of K induced by this steroid.

The influence of other adrenocortical steroids and ACTH on brain electrolytes has been studied by Woodbury (1954, 1963) in rats and by Timiras *et al.* (1954) in mice. The results may be summarized as follows In rats, chronic administration of ACTH, cortisone, cortisol, corticosterone, and dehydrocorticosterone had no effect on brain electrolytes in the doses used and for the period administered (28 days), although they markedly affected brain excitability. In mice, on the other hand, hydrocortisone increased brain excitability and markedly increased brain Na and Cl spaces. The increase in both Na and Cl spaces indicates either an effect of cortisol on the permeability of brain cells, such that both Na and Cl enter the cells without a net increase in intracellular Na concentration, or an effect on the glial tissue of brain The increase in brain Cl space induced by cortisol in mice suggests that extracellular space is influenced by this steroid; an increased permeability may occur, that is, the ground substance and glia may become more fluid. This possibility is borne out by the observation that cortisone affects growth and proliferation of glial cells in tissue cultures of cerebral cortex.

The influence of acute administration of cortisol (and DOC) on brain electrolyte metabolism has also been studied (Vernadakis and Woodbury, 1963a; Woodbury *et al.*, 1957). The results for DOC are the same as noted above for chronic injection of this steroid. However, the acute administration of cortisol differed from the chronic in that it increased brain intracellular Na concentration, decreased the brain Na ratio, and increased brain excitability. Thus the changes in brain electrolytes induced acutely by cortisol correlate with the observed changes in excitability. The results differ from those noted above for chronic cortisol treatment, in which no measurable changes in intra-

cellular brain electrolytes were noted. In this particular situation, the effect of chronic treatment on the connective tissue space of brain, or the shift of hydrogen ions across cell membranes which occurs during chronic cortisol administration and in Cushing's disease (Sprague *et al.*, 1951; Teabeaut *et al.*, 1950), may obscure the real electrolyte shifts and thereby modify brain excitability. Acute administration of DOC and cortisol affects brain amino acid as well as brain electrolyte metabolism; the relation between these two effects is discussed in Section III, C, 2.

The observation that chronic administration of cortisone and related steroids increases brain excitability without a significant change in brain electrolyte metabolism tends to confirm the conclusion that the central effects of DOC and adrenalectomy are mediated through an influence on electrolyte metabolism. If the electrolyte changes were secondary to the depressant or excitant effects of DOC and adrenalectomy, respectively, then cortisone, cortisol, and dehydrocorticosterone, three steroids which increase brain excitability, would be expected to cause an increase in brain cell Na concentration; this is not the case. Since cortisone and cortisol do not greatly influence electrolyte metabolism on chronic administration, the mechanism of their central action is still unexplained.

b. Sex steroids. The effects of sex steroids on electrolyte metabolism have been investigated by Woolley *et al.* (1960) and Woolley (1961). They found that the local concentrations of Na and K in cerebral cortex, cerebellum, and brain stem of intact adult male rats were not altered, whereas Cl concentration was significantly increased in all three areas on administration of testosterone propionate, estradiol dipropionate, or progesterone. Chloride space in cerebral cortex, brain stem, and cerebellum was significantly increased by progesterone, whereas testosterone increased only the Cl space in cerebral cortex. Thus the action of the sex steroids on brain excitability, unlike that of DOC but like that of cortisol, does not appear to be exerted through effects on electrolyte metabolism.

B. Effects on Cerebral Blood Flow and on Brain Oxygen and Glucose Consumption *in Vivo* and *in Vitro*

1. *Hypofunction*

a. Adrenal cortex. The effects of adrenalectomy on cerebral blood flow and cerebral oxygen consumption *in vivo* and on brain metabolism *in vitro* have been studied and reviewed by Hoagland and his collaborators (see Hoagland *et al.*, 1953a,b; Hoagland, 1954). On the assumption that the electrocorticographic changes in adrenalectomized

rats kept in good condition by sodium chloride therapy are the result of a decrease in cerebral circulation and an accompanying cerebral oxygen deprivation, these investigators studied "head blood flow" and oxygen consumption in adrenalectomized as compared to normal rats. A significant reduction of 61% in head blood flow was observed in adrenalectomized rats; there was a concomitant significant decrease of 43% in oxygen consumption. The administration of ACE, pregnenolone, or cortisone restored head blood flow and oxygen consumption to within normal limits in 2 hours; on the other hand, DOC had little restorative effect. In later experiments, using modified and more accurate techniques to measure cerebral blood flow, the same investigators (see Hoagland, 1954) found in adrenalectomized rats that cerebral blood flow was decreased 57% and oxygen consumption was reduced 18%; cortisone therapy fully restored normal values for both cerebral blood flow and oxygen consumption. Hoagland (1954) concluded "that the evidence supports the hypothesis that reduced brain oxygen consumption following adrenalectomy slows the electrocorticogram. The reduced oxygen consumption in turn appears to result from decreased cerebral circulation brought about by general reduction in cardiovascular tone and responsivity." In addition, it was noted that those agents which restore cerebral blood flow and oxygen consumption (ACE, pregnenolone, and cortisone) also restored the electrocardiogram to normal. Whether these effects of adrenalectomy on cerebral blood flow and metabolism in rats can be correlated with the increase in brain excitability produced by adrenalectomy requires further study. However, the fact that cerebral flow and oxygen consumption are reduced in adrenalectomized rats maintained on sodium chloride solution and that such maintenance therapy prevents a reduction in EST would suggest that brain excitability changes are unrelated to those two factors.

In contrast to conditions of hypercorticism (see below), the effect of *adrenal hypofunction* in man (Addison's disease) on cerebral blood flow has been little studied. Hafkenschiel *et al.* (1954) measured cerebral blood flow and oxygen consumption in 7 patients with severe essential hypertension before and after a reduction in blood pressure achieved by removal of 90% of the adrenal tissue, alone or combined with sympathectomy. They found that the mean values of oxygen consumption, jugular venous oxygen content, and jugular venous oxygen tension remained essentially unchanged and that cerebral blood flow increased slightly after such surgical procedures; the high cerebral vascular resistance associated with the severe hypertension was lowered toward the normal range. Whether cerebral blood flow and cerebral vascular resistance are decreased in patients with Addison's disease or would be

lowered by adrenalectomy in normal patients is not yet known. It is of interest, however, that Gordan *et al.* (1951) have observed a slightly decreased cerebral blood flow and oxygen consumption in patients with hypopituitarism; part of the decrease can certainly be attributed to the associated thyroid deficiency, since Gordan *et al.* also noted a decrease in brain oxygen consumption in patients with hypothyroidism; but whether the associated hypoadrenalism also contributes to the reduction is yet to be determined. These investigators state: "Despite our interest in the effects of corticoids upon cerebral metabolism, we have thus far lacked the courage to perform studies upon patients with Addison's disease. The tenuous metabolic balance of such patients precluded withdrawal of supportive therapy for a sufficient period of time to obtain data which could be considered as representative of a basal state."

Nevertheless, P. Scheinberg (cited by F. L. Engel, 1949) did measure cerebral blood flow, cerebral oxygen consumption, and cerebral glucose utilization in two cases of Addison's disease and in one case of hypopituitarism. Engel reported on one of these patients who was having what was clinically a perfectly characteristic hypoglycemic reaction at the time the cerebral blood flow was being studied, although the blood sugar level was 72 mg %; no change in cerebral blood flow, oxygen consumption, or glucose utilization was detected. When glucose solution and ACE were administered into the jugular vein, no change occurred in either cerebral blood flow or glucose utilization, but there was a slight decrease in oxygen consumption. Thus the meager data available indicate that cerebral blood flow and oxygen consumption are essentially unchanged in patients with adrenocortical insufficiency, a situation in agreement with the lack of effect of adrenalectomy on oxygen consumption of brain tissue *in vitro*, a topic now to be discussed.

The effects of *adrenalectomy* on cerebral oxygen consumption *in vitro* have been studied by several investigators. Although Himwich *et al.* (1934) noted a decrease in the oxygen consumption of minced brain obtained from adrenalectomized animals in extremis, most investigators have not observed any change in oxygen consumption of brain slices and homogenates (Bergen *et al.*, 1952; Crismon and Field, 1940; Tipton, 1939). Bergen *et al.* (1952) confirmed earlier observations that adrenalectomy does not influence oxygen consumption of brain slices and homogenates, but in addition found that the activity of cytochrome oxidase and succinic dehydrogenase in these same brain slices was normal. Whereas such data obtained *in vitro* agree with the lack of effect of adrenocortical insufficiency on cerebral blood flow and metabolism in man, there remains the fact that hypoadrenalism in

experimental animals appears to decrease cerebral oxygen consumption and blood flow. The discrepancy remains to be resolved.

b. Gonads. In vitro observations in animals. The effects of sex steroids on respiration of rat brain homogenates in glucose substrate have been reported by Gordan *et al.* (1951) and Gordan (1956). Their results are summarized as follows. The rate of oxygen uptake by the brain of the *immature castrated* rat is 32% higher than that of the normal rat. The brain of the castrated rat treated with testosterone respired at a rate which was more nearly normal that that of the untreated castrate, although the rate was still slightly higher than normal. When *castration* was performed upon *older* or malnourished male rats, an elevation of the oxygen consumption of the brain was not observed. Thus, age, degree of maturity, nutritional status, and possibly strain, are important factors in the production of these effects. The addition of testosterone *in vitro* further suppresses the oxygen uptake of brain homogenates from normal, castrated, or castrated-treated animals. The inhibition is far less in the castrates, however, than in either the treated castrates or the normal group.

2. *Hyperfunction*

a. Adrenocortical steroids. (1) *In vivo observations in man.* The effects of ACTH and cortisone on cerebral blood flow in man have been studied by several investigators. Schieve *et al.* (1951), who were the first to study the problem, observed that ACTH decreased cerebral blood flow in the 14 subjects tested and that this decrease was associated with an increase in cerebral vascular resistance, a slight increase in mean arterial blood pressure, and no change in cerebral metabolic utilization of oxygen or glucose. However, both Alman and Fazekas (1951) and Sensenbach *et al.* (1953) found no changes in cerebral blood flow or metabolism in subjects treated with ACTH or cortisone; similar negative results were obtained in 2 patients with Cushing's syndrome (Sensenbach *et al.* (1953)). Although an increase in cerebral vascular resistance was noted in the experiments of Sensenbach *et al.*, this was paralleled by an increase in mean arterial blood pressure; hence there was no change in cerebral blood flow. It must be concluded that the results of these studies provide no explanation for the mental, EEG, and brain excitability changes that occur during the administration of cortisone and ACTH.

The effects of DOC on cerebral blood flow and metabolism have been studied by Bentinck *et al.* (1951), Gordan *et al.* (1951, 1953), Gordan (1956), and Schieve and Wilson (1952). Although Bentinck *et al.* found that DOC produced no change in mean arterial blood pressure or cere-

bral blood flow, the steroid did cause a rise in the sugar concentration in cerebral venous blood over that in arterial blood. This increase indicates that DOC caused a liberation of sugar from the brain, probably galactose mobilized from brain cerebrosides (Gordan *et al.*, 1951, 1953), as discussed in Section III, C, 2. However, Schieve and Wilson (1952) were unable to confirm the above-described results; in their experiments, DOC did not cause an increase in sugar concentration in cerebral venous blood. The *in vivo* effects of sex steroids are discussed in Section III, B, 1, b.

(2) *In vitro observation in animals.* (See reviews by Gordan *et al.*, 1951, 1953; Gordan, 1956.) The effects of various *steroids* on the respiration of rat brain homogenates were studied by Gordan and Elliott (1947). DOC and progesterone and other nonadrenal steroids were compared with cholesterol with respect to their influence on oxidation of glucose, succinate, and pyruvate. The inhibition of respiration in the presence of glucose or pyruvate (Gordan *et al.*, 1951) produced by DOC and progesterone and the lack of effect produced by cholesterol ran parallel with the reported anesthetic potency of these steroids. However, these steroids had little effect upon the oxidation of succinate. In contrast to the inhibitory effect of DOC and progesterone on rat brain respiration, cortisol has been shown to increase the endogenous oxygen uptake of cerebral cortex slices removed from rats 6 hours after administration of cortisol (A. Vernadakis and D. M. Woodbury, unpublished observations, 1963). This is a time when a single dose of cortisol produces its maximal effect to decrease EST. The observations that DOC and progesterone decrease oxygen consumption and increase EST whereas cortisol and estradiol increase oxygen consumption and decrease EST suggest that brain excitability is closely related to oxidative metabolism of the brain.

The depression of brain respiration by DOC, as observed by Gordan and Elliott (1947), has been confirmed by Eisenberg *et al.* (1950), who found a linear relation between the logarithm of the dose and the percentage of inhibition of respiration. Hayano *et al.* (1950) also noted that DOC inhibited oxygen consumption of rat brain slices and homogenates in the presence of a variety of oxidizable substrates. However, the inhibition was not the result of an interference with the cytochrome c–cytochrome oxidase system since DOC did not block this system. In an earlier study, Tipton (1939) demonstrated that ACE and corticosterone depressed the oxygen consumption of rat brain tissue.

Gordan *et al.* (1951) have concluded that the locus of steroidal inhibition of oxidation of glucose or pyruvate, not reversed by methylene blue, is at the level of the dehydrogenases. They have pointed out

further that, since anesthetic agents are believed to produce their biological effects by inhibiting the utilization of carbohydrate at the cytochrome level, it appears that steroids have a different mode of action than do the usual anesthetics. The observations of Hayano *et al.* support these conclusions.

The oxygen consumption is high in brains removed from castrated male rats. If such rats are first treated with ACTH, DOC, testosterone, progesterone, or other steroids, the *in vitro* oxygen consumption of their brains is reduced to normal. The efficacy of ACTH in this respect indicates that the adrenal steroids released by it can also inhibit oxygen consumption of rat brains *in vivo*. The relation of these findings to carbohydrate, protein, and fat metabolism of brain is discussed in Section III, C.

C. Effects on Carbohydrate, Phosphorus, Protein, and Fat Metabolism

1. *Hypofunction*

a. Adrenal cortex. The influence of adrenocortical insufficiency on brain carbohydrate, protein, and fat metabolism has been little studied, despite the considerable research carried out on the animal as a whole and on discrete tissues other than the brain. Adrenal insufficiency in experimental animals and man is characterized by low blood glucose levels during fasting, depletion of liver but not of muscle glycogen, decreased urinary excretion of nitrogen, high respiratory quotient, increased sensitivity to insulin, and increased rate of oxidation of carbohydrate. Since marked changes in the metabolism of carbohydrate, protein, and fat occur in the animal with hypocorticism, it might be expected that the brain would also show these same alterations. Little work has been done on the brain to prove this possibility. It has been observed that the oxygen consumption and the respiratory quotient of brain tissue removed from animals with adrenal insufficiency are not different from those of normal animals. However, Hoagland's group found that cerebral blood flow and oxygen consumption are decreased in adrenalectomized rats (see above), and Gordan *et al.* (1951) showed that surgical hypophysectomy of a patient resulted in a decreased rate of oxygen consumption but an increased rate of glucose utilization by the brain. These observations are consistent with the suggestion that adrenalectomy increases the rate of oxidation of glucose by the brain, but they indicate also that oxidative metabolism is simultaneously inhibited. However, as mentioned earlier, patients with Addison's

disease exhibit no change in glucose utilization by brain. Much further work is necessary to resolve this problem.

Vaccari and Rossanda (1951) noted that the brains of adrenalectomized rats fasted for 32 hours and receiving sodium chloride solution to drink had lower concentrations of glycogen and slightly lower concentrations of total carbohydrate than did the brains of similarly treated intact rats. This again indicates an increased rate of utilization of carbohydrate by brain tissue as a result of adrenalectomy. However, unpublished experiments (Woodbury, 1963) have shown that adrenalectomized rats, whether on water or on sodium chloride solution to drink, exhibit no change in brain and muscle glycogen despite a marked reduction in liver glycogen. Sass-Kortsak (1944) also observed no effect of adrenalectomy on brain glycogen content. The glycogen content of rat brain is decreased by hypophysectomy and can be restored to normal levels by treatment with ACTH (Abood and Kocsis, 1950; Woodbury, 1963); whether this decrease is due to adrenocortical deficiency has not been established. More definitive studies on the carbohydrate metabolism of the brain under conditions of adrenocortical insufficiency are necessary before further conclusions are warranted.

The influence of hypoadrenalism on free amino acid concentration in brains of rats has been studied by Vernadakis and Woodbury (1963a); as might be expected from the fact that adrenalectomy increases protein synthesis in other tissues, the total free amino acid concentration of the brain was markedly decreased, with the exception that the concentrations of glutathione and cysteic acid were increased. The amino acids, γ-aminobutyric acid (GABA), glutamine, glutamic acid, taurine, valine, and cystine, were markedly decreased in concentration by adrenalectomy; aspartic acid concentration was unchanged. These observations suggest that brain is similar to other tissues with respect to protein and carbohydrate metabolism. The decrease in GABA is of interest in view of the recent suggestion that GABA plays a role in oxidative metabolism of the brain or may be an inhibitory mediator substance in the central nervous system (Bazemore *et al.*, 1957; Elliott and Florey, 1956). A decrease in GABA, as occurs in adrenalectomy, might be related to the increased brain excitability known to occur in adrenalectomized animals.

From the meager data available, it is evident that the brain of adrenalectomized animals oxidizes carbohydrate at a higher rate than normal and that protein synthesis is accelerated. No reports have come to the reviewer's attention concerning the *fat* metabolism of the brain in adrenocortical insufficiency.

b. Gonads. Even fewer data are available concerning the effects of gonadectomy on brain carbohydrate, phosphorus, protein, and fat metabolism than are available for the effects of adrenalectomy on these same parameters. Consequently, discussion of this aspect is unnecessary.

Summary: In summary of the cerebral metabolic effects of adrenocortical and gonadal insufficiency, it may be stated that the brain of adrenalectomized rats is deficient in its ability to pump sodium, and that a secondary defect in potassium metabolism is associated with this deficiency; the resulting brain electrolyte changes are associated with an increase in brain excitability. In addition, adrenal hypofunction results in a decreased cerebral metabolic rate, apparently related to the EEG alterations observed in adrenalectomized animals and in patients with Addison's disease. Finally, an increased utilization of carbohydrate and an increased synthesis of protein have been observed in the brain of the adrenalectomized animal. All the foregoing changes may very well be the cause of the variegated neurophysiological abnormalities occurring in the animal with adrenocortical hypofunction. The cerebral metabolic effects of gonadal insufficiency cannot be summarized because of the small amount of data currently available.

2. *Hyperfunction*

The influence of *adrenocortical steroids* and *ACTH* on *carbohydrate metabolism* of the brain has not been extensively studied. Much of the pertinent literature has been summarized by Gordan *et al.* (1951) and by Gordan (1956). The overall effect of ACTH and cortisone-like steroids is to inhibit the oxidation of glucose in the tissue and to increase the deposition of glycogen, presumably derived from increased gluconeogenesis from proteins. Brain seems to be no exception. Increased deposition of glycogen in brain as a result of treatment with small doses of cortisone, and with DOC to a lesser extent, has been reported by Vaccari and Rossanda (1951) in adrenalectomized rats; total carbohydrate content of brain was also increased by cortisone. After large (anesthetic) doses, Vaccari and Malaguti (1951) noted that DOC increased the glycogen and total carbohydrate content of rat brain, whereas cortisone and pregnenolone in large doses had no such metabolic effect and did not cause anesthesia. Since other anesthetic agents (ether, barbiturate) increase the glycogen and total carbohydrate content of the brain, it was concluded that the DOC-induced effect was the result of reduced carbohydrate utilization during anesthesia (Fregni *et al.*, 1953). Timiras *et al.* (1956) have demonstrated that small doses of cortisol increase the deposition of glycogen in brain, muscle, and liver of rats, and that this increase is associated with an elevation in blood

sugar concentration and an enhanced recovery from electroshock seizures. Additional findings of these investigators established a clear association between speed of recovery from postical depression and the level of blood sugar; pancreatectomized rats and intact rats treated with alloxan, glucagon, glucose and cortisol recovered rapidly and had high blood sugar levels, whereas insulin-treated rats recovered slowly and had low blood sugar levels. It was therefore concluded that the process of postical recovery is intimately associated with carbohydrate metabolism, particularly with brain glucose utilization; in contrast, the excitability process is associated with alterations in brain Na ratio. Since the blood is the main source of glucose for the brain (the glycogen stores being small), procedures or drugs that influence blood sugar concentration consequently affect brain metabolism and the process of recovery from postexcitatory depression.

The effect of cortisol on glycogen concentration of brain may be attributed, as it is in other tissues, to a dual effect of this hormone, namely inhibition of glucose utilization and acceleration of gluconeogenesis. Since glucose is the main source of energy in the brain *in vivo*, it can hardly be expected that the inhibitory action of cortisol on the rate of oxidation of glucose plays any important role in the increase in storage of glycogen observed in brain after administration of this hormone. Rather, it is suggested that cortisol increases brain glycogen concentration by modifying protein and amino acid metabolism so as to accelerate gluconeogenesis.

Gordan *et al.* (1951) reported that cerebral glycogen and cerebral galactose may be mobilized in man by DOC in some instances; this suggests that glycogen can serve as a source of rapidly available energy. Abood and Kocsis (1950) noted in rats that the brain glycogen content, reduced by hypophysectomy, could be restored to normal by ACTH. Thus brain glycogen seems to be markedly influenced by adrenocortical steroids, cortisone-like steroids increasing and DOC-like steroids decreasing brain glycogen. The decrease in glycogen produced by DOC is probably the result of a decrease in gluconeogenesis from protein. Indeed, Timiras and Woodbury (1957) have noted that DOC increases the concentration of many free amino acids in rat brain, a fact which might indicate a decrease in gluconeogenesis from amino acids.

Reports on the effects of *sex steroids* on *brain carbohydrate metabolism* are meager. Recent studies by Woolley (1961) have shown the following: estradiol administration produced no significant change in the glycogen concentrations of cortex, cerebellum, or brain stem; progesterone decreased glycogen in both the cortex and cerebellum, but had no effect on the brain stem. Estradiol plus progesterone,

testosterone, and methylandostenediol all increased cortex glycogen but did not alter the concentrations in the cerebellum and brain stem.

The effects of *ACTH* on *phosphorus metabolism* have been studied by Torda (1953, 1954) and Torda and Wolff (1952c, 1954) in the forebrain of mice and by Loeb *et al.* (1953) in the hypothalamus and the brain stem of monkeys. Torda and Wolff found that, after acute administration, ACTH increased the amount of phospholipid phosphorus and P^{32}-labeled phosphorus without altering other phosphorus-containing fractions of the brain (Torda, 1954; Torda and Wolff, 1954); it was concluded that ACTH increases brain phospholipid synthesis. However, Loeb *et al.* (1953) noted that chronic treatment with ACTH increased turnover of P^{32} in all brain phosphorus fractions (acid-soluble, phospholipid, pentosenucleic acid, and phosphoprotein), but did not change the total amount of phosphorus in any of these fractions; they concluded that ACTH increased metabolic activity of the brain areas examined. On the basis of this conclusion, and assuming that ACTH secretion is under control of the hypothalamus, Loeb *et al.* suggested that hypothalamic stimulation inhibits the secretion of ACTH, a suggestion also made by Roberts and Keller (1955). Reiss *et al.* (1949) studied the effects of hypophysectomy and ACTH on phosphorus metabolism of the central gray matter of rats and noted that hypophysectomy increased the P^{32} uptake of the total acid-soluble fraction of whole brain and that ACTH prevented the increased uptake. Phospholipid-P^{32} uptake, 125 hours after injection of the isotope, was also enhanced by hypophysectomy and to an even greater extent by ACTH. However, Kocsis (1956) found ACTH decreased phospholipid-P^{32} uptake in the brain, but the values were determined 1 hour after injection of the isotope. Reiss *et al.* concluded that the increased activity of the brain with regard to phosphorus metabolism in hypophysectomized rats may possibly be due to an enhanced carbohydrate utilization and that ACTH restores normal metabolism by a decrease in carbohydrate utilization. These observations are supported by the earlier results of Reiss and Rees (1947), who found that hypophysectomized and adrenalectomized rats exhibited an increase in hexokinase activity and anaerobic glycolysis and that the latter function was restored to normal by ACTH. However, neither hypophysectomy nor ACTH affected the oxygen uptake of slices of gray matter removed from rat brains.

The influence of *adrenocortical steroids* on *protein and amino acid metabolism* of brain has not been adequately studied, although their effect on other tissues has received considerable attention. If the data obtained on other tissues can be applied to brain, it would be expected

that ACTH and cortisone-like steroids would decrease the synthesis and increase the breakdown of proteins and accelerate gluconeogenesis, and that adrenalectomy would have the opposite effects. The observations of Loeb *et al.* (1953) that ACTH increased the turnover of P^{32} in the pentosenucleic acid fraction of the monkey hypothalamus might indicate an increase rather than a decrease in the rate of synthesis of cellular protein. However, direct determination of protein synthesis and breakdown under the influence of adrenocortical hormones is necessary before any conclusions are warranted.

The effects of *DOC* on brain amino acid metabolism have been studied more extensively. Gordan *et al.* (1953) observed that the conversion of ammonia and glutamic acid to glutamine in human brain was prevented or reversed by DOC. Timiras and Woodbury (1957) studied the influence of DOC on the free amino acid content of cerebral cortex in young and old rats and correlated these changes with alterations in brain excitability and electrolytes. The most striking findings were changes in the glutamic acid–glutamine system and aspartic acid. In line with the evidence presented by Gordan *et al.* (1953) the data obtained in the experiments of Timiras and Woodbury demonstrate that castration increases the conversion of ammonia and glutamic acid to glutamine and that DOC restores the normal relationship found in the intact controls. An interpretation of the changes noted in the other amino acids cannot be made at this time. The observations are also consistent with those reported by Gordan *et al.* (1951) that castration increases brain Q_{O_2} to normal values and the data suggest that increased oxygen consumption by brain results in increased utilization of glutamic acid and/or increased conversion of glutamic acid to glutamine, whereas DOC decreases such utilization and/or conversion.

Certain observations may now be summarized for purposes of correlation. Adult animals treated with DOC exhibit an increase in threshold for seizures, a decrease in brain Na concentration, an increase in brain glutamic and aspartic acid concentrations, and a decrease in brain glutamine. In contrast, young animals treated with DOC exhibit a decrease in threshold for seizures, an increase in brain Na concentration, and an increase in brain glutamic and aspartic acid concentrations. These facts suggest that glutamic acid and glutamine and aspartic acid are associated with brain electrolyte alterations and thereby influence brain excitability. Further experiments are necessary to evaluate this suggestion. It is of interest, moreover, that Terner *et al.* (1950) have associated the transport of K across brain cells with glutamic acid metabolism.

The effects of acute and chronic administration of DOC and cortisol

on brain amino acids have been measured in intact and adrenalectomized rats (Vernadakis and Woodbury, 1963a; Woodbury *et al.*, 1957). Cortisol (acute and chronic treatment) increased the concentration of most free amino acids in brain with the exception that the concentration of glutamine and GABA was decreased. DOC (acute and chronic treatment) had opposite effects from those of cortisone on glutamic acid, glutamine, and GABA. The decrease in brain concentration of GABA induced by cortisol is consistent with other evidence that the brain concentration of this amino acid is correlated with brain excitability (Woodbury and Esplin, 1959; Woodbury and Vernadakis, 1958). Thus the cortisol-induced increase in brain excitability is associated with a decrease in brain GABA, whereas the DOC-induced decrease in brain excitability is associated with an increase in this amino acid.

No data are available on the effect of the *sex steroids* on brain protein and amino acid metabolism, hence this aspect cannot be discussed at the present time.

The effects of *adrenocortical hormones* on *fat metabolism* particularly in brain, are not clear; much more information is needed. A single injection of ACTH in mice transiently increased the total phospholipid content of cerebral cortex and accelerated the turnover of P^{32} in this fraction; however, chronic treatment with ACTH, DOC, cortisone, hydrocortisone, progesterone, or 21-acetoxypregnenolone had no effect on total phospholipid concentration (Torda, 1954; Torda and Wolff, 1954). In contrast, Loeb *et al.* (1953) noted that chronic administration of ACTH in the monkey increased the turnover of P^{32} in the phospholipid fraction of the hypothalamus. The meager data suggest that ACTH increases synthesis and breakdown of brain phospholipid. The effects of cortisone and DOC on P^{32} turnover in phospholipids are not known. The relation of phospholipid metabolism to changes in brain excitability and electrical activity remains to be ascertained. The postulation by Bernheim (1952) that adrenocortical hormones are concerned in the metabolism of brain linolenic acid requires confirmation.

The effects of *sex steroids* on brain *fat* metabolism also have not been studied sufficiently to warrant inclusion in this chapter.

IV. Steroidal Regulation of Brain Excitability

Evidence has been reviewed previously (Woodbury, 1958) that adrenocortical secretion of hormones regulating carbohydrate and electrolyte metabolism is under control of the central nervous system; cortisol secretion is regulated via the hypothalamic–adenohypophyseal system and aldosterone secretion via some area in the diencephalon. Also, evidence is presented in earlier sections of the chapter that the

adrenocortical steroids in excessive amounts cause marked changes in brain excitability; cortisol-like steroids increase excitability, whereas DOC-like steroids decrease excitability. Whether such effects of the adrenocortical steroids on brain excitability operate under physiological and pathological conditions remains to be elucidated. However, the following observations suggest a physiological role of the adrenocortical steroids in regulation of brain excitability. (1) In rats (Guillemin *et al.*, 1959; McCarthy *et al.*, 1960) and in mice (Halberg *et al.*, 1959), the corticosterone level in plasma exhibits a diurnal variation whereas the "cortisol-like material" level does not (McCarthy *et al.*, 1960). (2) In rats (Woolley and Timiras, 1962c), the EST also exhibits a diurnal variation, the time course of which parallels that of the corticosterone rhythm. (3) In mice (Halberg *et al.*, 1955), susceptibility to audiogenic seizures parallels the time course of the corticosterone concentration and varies with time, and the plasma "cortisol-like material" (presumably cortisol itself) concentration does not; the ratio between the two (B/F) probably determines the level of excitability in the CNS as suggested by Woodbury (1954) and Woodbury *et al.* (1957). At the low points in the cycle the B/F ratio is 0.65 for males and 1.39 for females whereas at the high point the ratio is 3.35 for males and 2.01 for females (McCarthy *et al.*, 1960). Since cortisol produced a marked increase in excitability whereas corticosterone had little effect, it would be expected that when the B/F ratio is low excitability would be increased and that when it is high excitability would be decreased. The experiments of Halberg *et al.* (1955) and Woolley and Timiras (1962c) confirm this expectation. Definitive proof, however, that the diurnal changes in brain excitability are a result of the diurnal changes in B/F ratio awaits experiments in which EST and plasma B/F ratio are determined in adrenalectomized animals maintained on a constant dose of adrenocortical steroids. In such animals the diurnal variation in EST should be abolished if the B/F ratio is a determinant of brain excitability.

Various workers have delineated three separate functional roles of the adrenal cortex—"permissive," "active," and "homeostatic"—and there is evidence that all three are concerned with the regulation of central nervous system functions. The term "permissive" ("supporting") has been used by Ingle (1952, 1953) to characterize the relationship of the adrenocortical hormones to certain metabolic responses which fail to become overt in animals with adrenocortical insufficiency, but which reappear when doses of adrenocortical hormones adequate to maintain a state of eucorticism are administered. The reappearance of such metabolic responses cannot be attributed to an increase in secretory activity of the adrenal cortex, because even the adrenalectomized

animal can be restored to normal by appropriate therapy. The adrenocortical steroids thus "permit" but do not cause a given effect to occur. An example of the permissive role of the adrenocortical steroids in nervous system function is the observation of Porter (1954) that the enhanced electrical activity in the hypothalamus induced by epinephrine is annulled by adrenalectomy and restored by maintenance doses of cortisone or cortisol; excessive amounts of these steroids do not augment the effect of epinephrine.

However, many of the central nervous system effects of adrenocortical steroids cannot be ascribed to their permissive role, and an "active" role must be evoked. For example, the increase in brain excitability induced by thyroxine in intact rats is almost twice as great as that in adrenalectomized rats (Timiras *et al.*, 1955); the attenuated effect of thyroxine on brain excitability in the adrenalectomized rat has been attributed to the lack of even a minimal amount of adrenocortical steroids and to the consequent decrease in responsivity of the brain. This possibility is in accord with the "permissive" effect just described. If this proposal is correct, then maintenance doses of adrenocortical steroids given to adrenalectomized, thyroxine-treated rats would be expected to restore the normal response to thyroxine, and brain excitability should be markedly increased. The experiment was performed and the expected result did not occur. Instead, ACE not only prevented the usual moderate enhancement in excitability which thyroxine induced in adrenalectomized rats, but actually prevented any increase in excitability. These results indicate that the adrenal cortex activity participates in the response of the central nervous system to thyroxine. Approximately half of the effect of large doses of thyroxine on the nervous system is the result of adrenocortical stimulation; the other half is the result of a direct excitatory action of thyroxine itself on the brain.

The above experimental results also illustrate another aspect of the effect of adrenocortical hormones on nervous system function, namely, their ability to "normalize" or regulate brain function when it has deviated from normal (Woodbury, 1954; Woodbury *et al.*, 1957). The concept of a "normalizing" or regulatory role of the adrenal cortex is also based on additional observations (Woodbury, 1952a, 1954; Woodbury *et al.* 1957), as follows: (1) Adrenocortical steroids with an oxygen at C-11 antagonize the decrease in brain excitability induced by DOC. (2) ACTH and 11-dehydrocorticosterone, given chronically partially antagonize the increase in brain excitability induced by cortisone. (3) A single dose of corticosterone prevents to a large extent the decrease in excitability induced by DOC and the increase in excita-

bility induced by cortisol. (4) DOC, cortisol, and many other agents cause a greater change in brain excitability in adrenalectomized than in intact animals. (5) Experimental studies of the effects of salt and water loads on brain excitability of intact and adrenalectomized animals demonstrate that such loads alter excitability of the adrenalectomized rat much more than that of intact animals. (6) Certain centrally acting drugs which alter brain excitability stimulate the hypothalamic–adenohypophyseal system to release ACTH, and the adrenocortical hormones secreted as a result of such stimulation tend to antagonize the alteration in brain excitability induced by the drug. These data suggest that some secretory product of the adrenal cortex, released as a result of ACTH stimulation, regulates central nervous system excitability in such a way as to restore an abnormally increased or decreased excitability to normal. Evidence that the regulatory hormone of the adrenal cortex, at least in the rat, is corticosterone has been summarized previously (Woodbury, 1954) and will not be discussed here. It can therefore be concluded that the adrenocortical hormones have the three influences on central nervous system function, as described above: a permissive influence in that normal brain excitability is not maintained in the absence of adrenocortical hormones, an active influence in that the concentration and pattern of steroids in the blood exert a direct effect on brain excitability, and a regulatory influence in that factors which alter brain excitability also cause adrenocortical steroids to be released which tend to counteract the original change in excitability. The possible metabolic factors involved in the regulation of brain excitability by the adrenocortical hormones have been discussed by Woodbury (1958) and will not be considered here.

Evidence that the female sex steroids play a physiological role in the regulation of brain excitability is provided by the experiments of Woolley and Timiras (1962c). They found that the EST and MES pattern of female rats varied with the estrous cycle. The threshold for minimal seizures was highest during diestrus, lower during proestrus, and lowest during estrus. The duration of the tonic flexor component was longest during diestrus and shortest during estrus. It is likely that the increased brain excitability during estrus is due to the high plasma levels of estradiol during this period. Estradiol-17β, as described in a previous section (II, B, 2), markedly increases brain excitability. In the human female, increased tension and nervousness often appear prior to menstruation. This premenstrual tension is probably related to an increase in excitability of the CNS as a result of the rapid fall in the plasma progesterone level and the rise in the estradiol level which occur at this time. Progesterone raises and estradiol lowers the EST (see

Section II, B, 2, a) and the combination of a rapid decrease in progesterone and a rise in estradiol-17β levels in the plasma induces hyperexcitability of the CNS. It appears clear from this brief discussion that the sex as well as the adrenocortical steroids play a physiological rọle in regulating the level of excitability in the central nervous system.

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Chapter 2

The Relation between the Structure and Physiological Activity of Progestational Steroids

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I. Introduction

The isolation and identification of progesterone advanced our knowledge of the physiological function of this steroid hormone, but unfortunately did not significantly advance practical therapeutics in this field (Fels and Slotta, 1930, 1931; Allen, 1932; Ferold and Hisaw, 1932; Butenandt *et al.*, 1934; Slotta *et al.*, 1934a,b; and Allen and Wintersteiner, 1934). The discovery of ethisterone (17α-ethynyl-17β-hydroxyandrost-4-en-3-one) (Inhoffen *et al.*, 1938), a weak orally active progestational hormone, was of some practical importance. The rapid development of the field, however, dates from the synthesis of norethisterone (17α-ethynyl-17β-hydroxyestr-4-en-3-one) and the discovery of its high oral potency and its practical therapeutic efficiency (Birch, 1950a,b; Djerassi *et al.*, 1954; and Ringold *et al.*, 1956b).

The demonstration that it was possible to synthesize a steroid of high oral activity and of considerable clinical value stimulated the search for other valuable agents of this class. This chapter will review in some detail the various synthetic progestational steroids that have become available during the past few years, particularly with respect to molecular structure and function.

II. The Relation between the Position of the Double Bond and Progestational Action

The progestational activity of derivatives of progesterone (pregn-4-ene-3,20-dione) is usually associated with the presence of a Δ^4 double bond. Varying the position of the double bond as shown in Table I changes the progestational activity as indicated. Reduction of the Δ^4 bond leads to 5β-pregnane compounds devoid of progestational activity (Fig. 1). Although 3-alkoxypregna-3,5-dien-20-one lacks a Δ^4 double bond, it does show progestational activity. Since this compound has a conjugated double bond, it is possible that a resonant isomer exists as indicated in Fig. 2, and that the $\Delta^{3,5}$ structure is similar to the active $\Delta^{4,6}$ structure.

Compounds having a Δ^{16} double bond in addition to the Δ^4 double bond (pregna-4,16-diene-3,20-dione) do not have progestational activity. This lack of activity may be due in part to the steric configuration of the 17β-acetyl group. This subject will be discussed in Section III.

Compounds having one or two double bonds in addition to the Δ^4 double bond may have progestational activity (Table I), except for those having three double bonds in ring A, such as the inactive steroid 3-hydroxy-19-norpregna-1,3,5(10)-trien-20-one.

TABLE I

THE EFFECT OF VARYING THE POSITION AND NUMBER OF DOUBLE BONDS IN THE PROGESTERONE MOLECULE ON PROGESTATIONAL ACTIVITY DETERMINED BY THE SUBCUTANEOUS CLAUBERG ASSAY

Compound	Position of double bond	Relative potency, progesterone = 1
Pregn-4-ene-3, 20-dione	Δ^{4}	1
5α-Pregn-1-ene-3,20-dione	Δ^{1}	Inactive
Pregna-1,4-diene-3,20-dione	$\Delta^{1,4}$	Slightly < 1
Pregna-3,5-dien-20-one, 3-alkoxy-	$\Delta^{3,5}$	Active
Pregn-4-en-20-one, 3β-hydroxy-	Δ^{4}	1
Pregna-4,6-diene-3,20-dione	$\Delta^{4,6}$	0.4–0.5
Pregna-4,9(11)-diene-3,20-dione	$\Delta^{4,9(11)}$	0.25–0.5
Pregna-4,11-diene-3,20-dione	$\Delta^{4,11}$	3
Pregna-4,16-diene-3,20-dione	$\Delta^{4,16}$	Inactive
Pregn-5-en-20-one, 3β-hydroxy-	Δ^{5}	Inactive
Pregna-5,7-dien-20-one, 3β-hydroxy-	$\Delta^{5,7}$	Inactive
Pregna-5,7,9(11)-trien-20-one, 3β-hydroxy-	$\Delta^{5,7,9(11)}$	Inactive

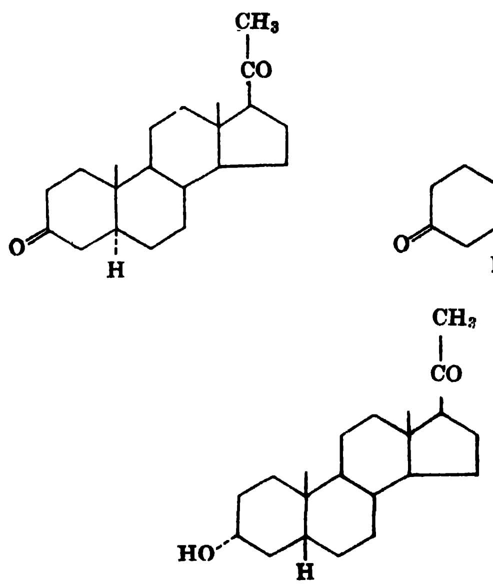


FIG. 1. Inactive pregnane derivatives in which the Δ^4 double bond has been reduced.

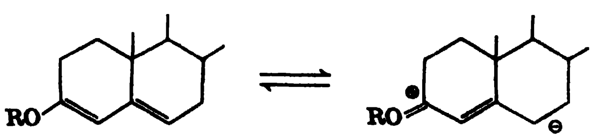


FIG. 2. Possible resonant isomer of 3-alkoxypregna-3,5-dien-20-one.

Steroids with Δ^1 or Δ^6 double bonds in addition to the Δ^4 double bond tend to be more active than the parent compounds. This is especially true when the compounds are studied by the oral route. When double bonds are introduced into 17α-acetoxyprogesterone (17α-hydroxypregn-4-ene-3,20-dione acetate) and (or) when substitutions are made at position 6 in the molecule, increased progestational activity results, as indicated in Table II.

Table II indicates that the progestational activity of the derivatives of 17α-acetoxyprogesterone is related to the number and position(s) of double bonds in the following manner: $\Delta^{4,6} > \Delta^{1,4,6} > \Delta^{1,4} > \Delta^4$. This

TABLE II

THE PROGESTATIONAL ACTIVITY OF 17α-ACETOXYPREGN-4-ENE-3,20-DIONE DERIVATIVES DETERMINED BY THE ORAL CLAUBERG ASSAY

	Relative potency, norethisterone = 1			
	Position(s) of unsaturation			
6α-Substituent	Δ^4	$\Delta^{1,4}$	$\Delta^{4,6}$	$\Delta^{1,4,6}$
H	0.07	—	—	1–2
F	1	6	15	8
Cl	2–3	8	35–50	35
Br	1	6	—	—
CH_3	4–5	8	12	10

rule of relative potency seems to hold for other derivatives of progesterone. In the 16α-methyl-17α-acetoxyprogesterone (17α-hydroxy-16α-methylpregn-4-ene-3,20-dione acetate) series, however, the relationship is changed as follows: $\Delta^{1,4,6} > \Delta^{4,6} > \Delta^{1,4} > \Delta^4$. Retroprogesterone (9β,10α-pregn-4-ene-3,20-dione), a novel steroid having high progestational activity, is less active than the Δ^6 unsaturated derivative (9β,10α-pregna-4,6-diene-3,20-dione). The general rule is followed in the case of 9α-chloro-11β-fluoropregn-4-ene-3,20-dione, in which the introduction of a Δ^1 double bond results in a compound with increased progestational activity, but is not followed in the case of 9α,11β-dichloropregn-4-ene-3,20-dione, where the introduction of a Δ^1 double bond results in decreased activity.

Although progestational activity is usually associated with the presence of a Δ^4 double bond, the isopropylidenedioxy derivatives of pregn-5-en-20-one elicit progestational activity when administered

orally. The high oral activity of steroids in this series may be due to the facile transformation of the Δ^5 to the Δ^4 double bond by the low pH in the stomach. Similar exceptions to the Δ^4 double bond rule have been observed for the derivatives of 19-nortestosterone (17β-hydroxyestr-4-en-3-one), including: 17β-hydroxy-17α-methylestr-5-en-3-one, 17α-ethyl-17β-hydroxyestr-5-en-3-one, and 17α-ethynyl-17β-hydroxyestr-5-en-3-one, which have a Δ^5 double bond, and 17β-hydroxy-17α-methylestr-5(10)-en-3-one, 17α-ethyl-17β-hydroxyestr-5(10)-en-3-one, 17α-ethynyl-17β-hydroxyestr-5(10)-en-3-one, and 17β-hydroxy-17α-propylestr-5(10)-en-3-one, which have a $\Delta^{5(10)}$ double bond. Iriarte *et al.* (1959) suggested that the Δ^5 double bond was changed to Δ^4 as a result of the compounds' being administered orally.

In summary, it is suggested that a Δ^4 double bond with high electric density between positions 4 and 5 is required for progestational activity.

III. The Relation between the Steric Configuration of the 17β-Acetyl Radical and Progestational Activity

The 17β-acetyl radical of progesterone is united to ring D of the steroid nucleus by a single bond so that in principle it can rotate freely (Fig. 3). However, the position of the 17β-acetyl radical is

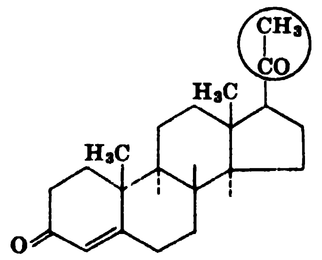


FIG. 3. Structural configuration of progesterone (17β-acetyl radical indicated by encircled area).

influenced by the steric hindrance of the angular methyl group attached to carbon 13, which causes the acetyl radical to lie in the same plane as that of the steroid nucleus. If one were to look at the steroid nucleus from above, as indicated in Fig. 4, the position of the acetyl radical would appear as indicated by either A or B. The angular methyl group attached to carbon 13 is indicated by the circled area. According to the study of the optical rotatory dispersion of Djerassi (1960), and the calculation of the dipole moment of Allinger and Da Rooge (1961) and others, the proper configuration of the 17β-acetyl radical of progesterone is that indicated by Fig. 4A.

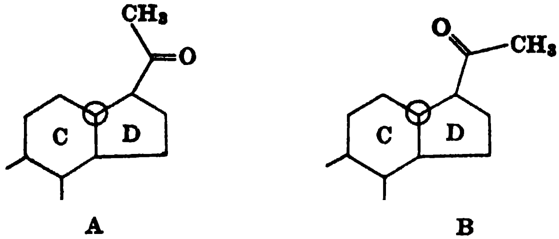


FIG. 4. Possible positions that the 17β-acetyl radical of progesterone may take.

That the 17β-acetyl radical of progesterone cannot rotate freely, primarily because of the interference of the angular methyl group, is an important factor in the ability of the compound to elicit progestational activity. The authors note three points (see Sections A–C below) as a basis for this supposition.

A. THE INFLUENCE OF THE ANGULAR METHYL GROUPS

The position of the 17β-acetyl radical is influenced primarily by the angular methyl group at position 13. When this methyl group is removed, the resulting compound, 18-norprogesterone (18-norpregn-4-ene-3,20-dione) has 0.25 the progestational activity of progesterone. In addition, the activity of 19-norprogesterone (19-norpregn-4-ene-3,20-dione) is 4–8 times greater than that of progesterone. (The activity increases when the steric hindrance of the plane of ring B against the Δ^4 double bond is diminished.)

18,19-Dinorprogesterone (18,19-dinorpregn-4-ene-3,20-dione) has 0.1 the progestational activity of progesterone. This lower activity is due to the fact that the 17β-acetyl radical is able to rotate freely. 17β-Methyl-isoprogesterone (17β-methyl-isopregn-4-ene-3,20-dione), in which the acetyl radical is in the α-position rather than the β-position as with progesterone, has no progestational activity; however, 18-nor-isopregn-4-ene-3,20-dione has 0.1 the progestational activity of progesterone as a result of the influence of the absence of carbon 18.

B. THE INFLUENCE OF 17α-SUBSTITUTION

The activity of various 17α-substituted progesterones is shown in Table III. Although 17α-hydroxyprogestesone (17α-hydroxypregn-4-ene-3,20-dione) had no activity, the 17α-hydroxy esters of progesterone were more active than progesterone. This may be due to the 17α-hydroxy group of 17α-hydroxyprogesterone becoming linked with the

TABLE III

THE EFFECT OF 17α-SUBSTITUTION ON THE PROGESTERONE MOLECULE DETERMINED BY THE SUBCUTANEOUS CLAUBERG ASSAY

Compound	17α-Substituent	Relative potency, progesterone = 1
Pregn-4-ene-3,20-dione, 17α-hydroxy-	OH	Inactive
Pregn-4-ene-3,20-dione, 17α-methyl-	CH_3	2
Pregn-4-ene-3,20-dione, 17α-bromo-	Br	2
Pregn-4-ene-3,20-dione, 17α-acetoxy-	$O \cdot CO \cdot CH_3$	7
Pregn-4-ene-3,20-dione, 17α-3-(*p*-hexanoxy-phenyl)-propyloxy-	$O \cdot CO \cdot R$	Active
Pregn-4-ene-3,20-dione, 17α-propyloxy-	$O \cdot CO \cdot R$	Active
Pregn-4-ene-3,20-dione, 17α-butoxy-	$O \cdot CO \cdot R$	Active
Pregn-4-ene-3,20-dione, 17α-pentoxy-	$O \cdot CO \cdot R$	Active
Pregn-4-ene-3,20-dione, 17α-heptanoxy-	$O \cdot CO \cdot R$	Active
Pregn-4-ene-3,20-dione, 17α-hexanoxy-	$O \cdot CO \cdot R$	Active
Pregn-4-ene-3,20-dione, 17α-octanoxy-	$O \cdot CO \cdot R$	Active

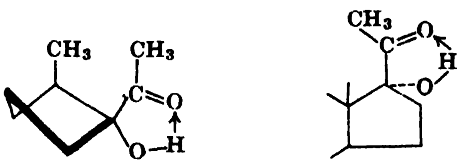
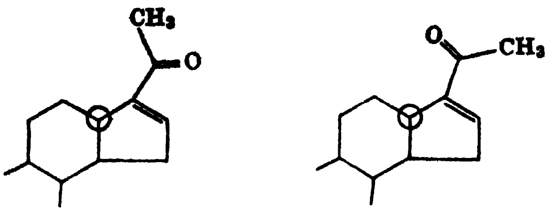


FIG. 5. Rotation of the 17β-acetyl radical due to the linkage of the ketone of the radical with the 17α-hydroxy group.

ketone of the acetyl radical, causing the acetyl radical to rotate from a horizontal to a vertical position with respect to the steroid nucleus (Fig. 5). Extension of this hypothesis permits one to see why it is that esterification of the 17α-hydroxy group results in compounds having activity.

C. THE INFLUENCE OF ADDITIONAL DOUBLE BONDS

Δ^{16}-Dehydroprogesterone was inactive in spite of the presence of a Δ^4 double bond (see Section II). The introduction of the Δ^{16} double bond into the 5-carbon ring D causes the 5 carbons of the ring to lie in approximately the same plane and also causes the 17β-acetyl radical to lie in the same plane as that of ring D. Also, since Δ^{16} dehydroprogesterone is the conjugated form, the 17β-acetyl radical assumes the position indicated in Fig. 6B rather than in Fig. 6A, so that it is in the reverse position of that previously indicated for progesterone (Fig. 4A) and therefore is inactive.



A B

FIG. 6. Possible positions that the 17β-acetyl radical of Δ^{16}-progesterone may take.

For the above three reasons it is believed that the stereochemical position of the 17β-acetyl radical is an important factor among others determining the ability of progesterone to elicit progestational activity.

IV. The Relation between Structural Changes and the Activity of Progesterone

A. *A*-NORPROGESTERONE

A-Norprogesterone, the structural configuration of which is shown in Fig. 7, has neither progestational nor anti-progestational activity.

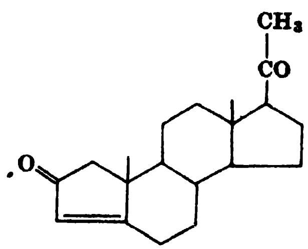


FIG. 7. The structural configuration of *A*-norprogesterone.

It has only been found to have anti-androgenic activity.

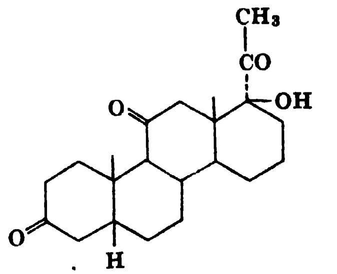
Substitution on carbon 2 results in reduced progestational activity. This may be inferred from the fact that 16α-methylprogesterone (16α-methylpregn-4-ene-3,20-dione) has approximately the same progestational activity as progesterone, whereas 2α,16α-dimethylpregn-4-ene-3,20-dione is inactive. The latter compound does have anti-inflammatory activity, however.

It is assumed, therefore, that *A*-norprogesterone has no progestational activity for the following three reasons: (1) The double bond occurs at carbon 3 rather than carbon 4. (2) It has a ketone group attached to carbon 2. (3) Ring A has five carbons rather than six carbons.

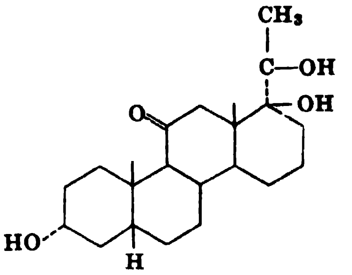
The 1,3,5(10)-triene form (3-hydroxy-19-norpregna-1,3,5(10)-trien-20-one), which has six carbons in ring A, also has no activity. This is due to the fact that the double bonds of ring A cause the carbons of the ring to lie in the same plane (flat ring). As was previously explained, it is necessary to have the cyclohexane form of ring A in order for progesterone to elicit progestational activity.

B. *D*-HOMOPROGESTERONE

Clinton *et al.* (1958) studied a series of *D*-homoprogesterone derivatives. None of them had progestational activity, in fact, two of the compounds elicited anti-progestational activity. These compounds are shown in Fig. 8. It is reasonable to expect that this *D*-homo series of



17aβ-Hydroxy-*D*-homo-17aα, 5β-pregnane-3,11,20-trione



3α,17aβ,20-Trihydroxy-*D*-homo-17aα, 5β-pregnan-11-one

FIG. 8. Two derivatives of *D*-homoprogesterone that showed anti-progestational activity.

compounds would have no progestational activity because it lacks a Δ^4 double bond, because the acetyl radical is in the 17α rather than the 17β position, and because they have a hydroxy group in position 17. Although several reports have been written concerning the *D*-homo form of progesterone, it is difficult to study the influence of the *D*-homo form itself because the compounds are quite different from progesterone.

It is interesting, however, that certain compounds in the series have anti-progestational activity. A comparison of compounds having anti-progestational activity and compounds having progestational activity should enable one to obtain a clue as to what structural phenomenon is the key to each type of activity.

C. 18-, 19-, and 21-Norprogesterone

18-Norprogesterone has already been discussed in Section III. The importance of the stereochemical position of the 17β-acetyl radical was emphasized. It is a strange fact that testosterone and 19-nortestosterone derivatives (Fig. 9) that have no 17β-acetyl radical show remarkable

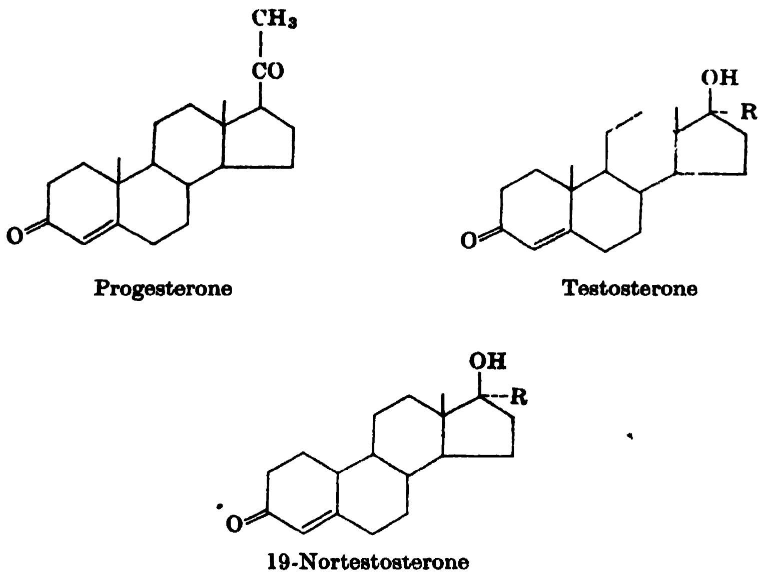
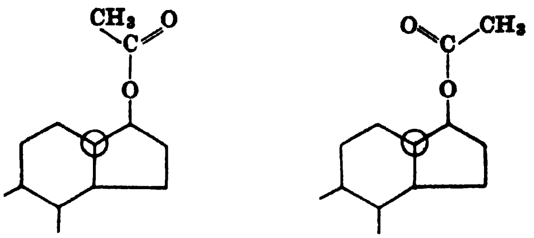


Fig 9 Comparison of the structures of progesterone, testosterone, and 19 nor testosterone derivatives (R = alkyl)

progestational activity—an activity that cannot be explained, as previously noted.

It was desirable, therefore, to look for similarities in the structures of the compounds discussed in Section III and the norprogesterones. The stereochemical position of the 17β-acetoxy group is influenced by the angular methyl group (carbon 18) and the alkyl radical in the 17α-position as indicated in Fig. 10. It is thought that the actual stereochemical position of the 17β-acetoxy group is that indicated by Fig. 10A rather than by Fig. 10B, assuming that, when the 17α-hydroxy group is acetylated, testosterone and 19-nortestosterone show progestational activity.



A B

FIG. 10. Possible positions that the 17β-acetoxy group of acetylated testosterone or 19-nortestosterone may take.

In general, the 19-nor derivatives of progesterone and testosterone have greater progestational activity than the parent compounds. 19-Norprogesterone (19-norpregn-4-ene-3,20-dione) is 4–8 times more active than progesterone. When administered orally, norethisterone (17α-ethynyl-17β-hydroxyestr-4-en-3-one) has 5 times more activity than ethisterone (17α-ethynyl-17β-hydroxyandrost-4-en-3-one). In general, 19-demethylation increases progestational activity.

When a hydroxy group is substituted for a hydrogen in the methyl group at position 19 of progesterone (19-hydroxypregn-4-ene-3,20-dione), the progestational activity decreased to 0.1 that of progesterone. Also, when a hydroxy group was introduced into position 10 of norethisterone, yielding 10β,17β-dihydroxy-17α-ethynylestr-4-en-3-one, the activity was decreased to 0.25 that of the latter steroid. The following relative activities are indicated: norethisterone > 10β-hydroxynorethisterone = ethisterone. Stucki (1958) and others have found 19-norethisterone (17α-ethynyl-17β-hydroxyestr-4-en-3-one) to be inactive in pregnancy maintenance tests.

The fact that the introduction of a methyl group in position 10β resulted in decreased progestational activity in the 19-nor derivatives may be explained by the stereochemical hindrance caused by such a methyl group. In this case the Δ^4 double bond influences the position of the 10β methyl group.

In general, 6α-substituted compounds are more active than 6β-substituted steroids (Kincl, 1961) because of the influence of the 6β substituents on the Δ^4 double bond. On this basis it seems that progestationally active steroids should not be hindered in the β direction. Ringold (1961), however, observed that this is true only when the substituent in the β position is located near the Δ^4 double bond. A similar situation exists with respect to retro- and isoprogesterone, which are influenced by the position of the angular methyl group in position 18. Additional studies with 8β- and 15β-substituted compounds are indicated.

21-Norprogesterone (3-oxo-21-norpregn-4-en-20-al) has weak progestational activity. Eliminating the 21-methyl group enables the 17β-aldehyde group to rotate more freely in a manner similar to that of the 18-nor compounds. The 21-methyl group is therefore not itself related to progestational activity, but it influences the stereochemical configuration of the ketone group attached to carbon 20, and thereby influences progestational activity indirectly.

Another possible explanation for the weak activity shown by 21-norprogesterone is the possibility that acetylation of the 17β-hydroxy group of testosterone or 19-nortestosterone derivatives produces the progestationally active form shown in Fig. 11A, whereas such acetylation of 21-norprogesterone does not take place, as seen in Fig. 11B.

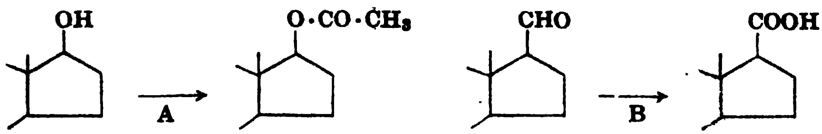


FIG. 11. Acetylation of testosterone and 19-nortestosterone takes place, yielding the progestationally active form (A). Acetylation of 21-norprogesterone does not take place, as seen in (B). The resulting compound is progestationally inactive.

This hypothesis may be extended to the 21-substituted compounds (Table IV). It can be seen from Table IV that increasing the molecular weight of the substituents decreases the progestational activity. This may be due to the fact that the direction of the 17β-acetyl radical may

TABLE IV

THE EFFECT OF 21-SUBSTITUTION IN THE PROGESTERONE MOLECULE ON PROGESTATIONAL ACTIVITY DETERMINED BY THE SUBCUTANEOUS CLAUBERG ASSAY

Compound	21-Substituent	Relative potency, progesterone = 1
Pregn-4-ene-3,20-dione	H	1
Pregn-4-ene-3,20-dione, 21-fluoro-	F	1–3
Pregn-4-ene-3,20-dione, 21-chloro-	Cl	Inactive
Pregn-4-ene-3,20-dione, 21-bromo-	Br	Inactive
Pregn-4-ene-3,20-dione, 21-hydroxy-	OH	0.1–0.15
Pregn-4-ene-3,20-dione, 21-methyl-	CH_3	0.15
21-Norpregn-4-en-20-al, 3-oxo-	Nor	Weak

be changed by the substituents attached to carbon 21. An exception to this generality, however, is the case of fluorine, which produces increased activity when attached to carbon 21.

D. Retro- and Isoprogesterone

The configuration of natural progesterone is shown in Fig. 3. Note that rings A/B, B/C, and C/D are located in the *trans* position in relation to each other, that hydrogens are located in the 8β, 9α, and 14α positions, and that methyl groups are located in the β position on carbons 10 and 13. Compounds having stereochemical configurations different from that of natural progesterone have been reported, however. These compounds are listed in Table V together with their progestational activity.

TABLE V

The Progestational Activity of Retro- and Isoprogesterone Derivatives Determined by the Subcutaneous Clauberg Assay

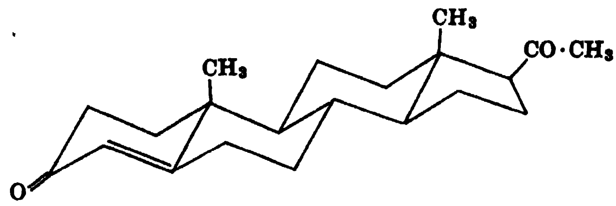
Compound	Stereochemical configuration	Relative potency, progesterone = 1
8α,9α-Pregn-4-ene-3,20-dione	8α,9α	0.25–0.5
9α,10α-Pregn-4-ene-3,20-dione	9β,10α	5
9β,10α-Pregn-4-ene-3.20-dione, 17α-hydroxy-, acetate[a]	9β,10α	28
14β,17α-Pregn-4-ene-3,20-dione	14β,17α	Inactive
19-Nor-14β-pregn-4-ene-3,20-dione[b]	14β,17β	8
19-Nor-14β,17α-pregn-4-ene-3,20-dione	14β,17α	8

[a] 17α-Acetoxyprogesterone has 7 times the progestational activity of progesterone. The 9-iso form shown here has 28 times the activity of progesterone; therefore, the 9-iso form has 4 times the activity of 17α-acetoxyprogesterone.

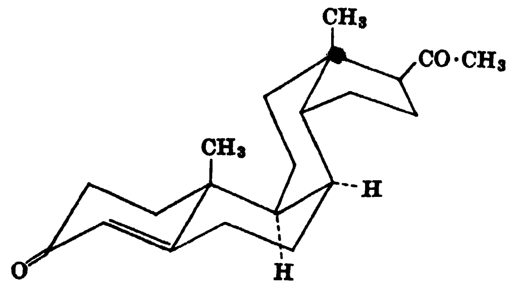
[b] 19-Norprogesterone has 4–8 times the progestational activity of progesterone. The 14β,17β and 14β,17α forms, therefore, have approximately the same activity as 19-norprogesterone.

Assuming that all the 6-carbon rings have the chair form of stereochemical configuration, the compounds listed in Table V may be depicted as shown in Fig. 12.

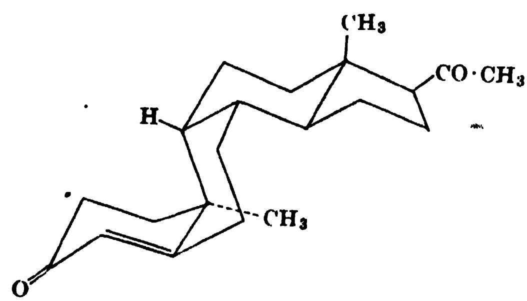
Since the B/C, C/D *cis* forms have progestational activity, it is not necessary to have the complete steroid molecule to obtain progestational activity. The most important determinants of progestational activity are the attached radicals and the "central core" of the steroid



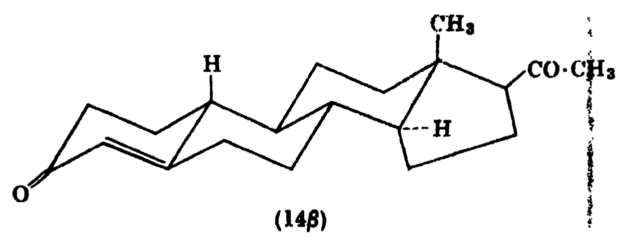
Progesterone



(8α, 9α)
8α,9α-Pregn-4-ene-3,20-dione



(9β,10α)
9β,10α-Pregn-4-ene-3,20-dione



(14β)
19-Nor-14β-pregn-4-ene-3,20-dione

FIG. 12. The stereochemical configuration of retro- and isoprogesterone derivatives.

molecule thought to be responsible for progestational activity. The entire steroid molecule is only of secondary importance in that it provides distance between areas of electrical density and proper steric configuration. If the nature of the active center or active substituent groups of the steroid molecule necessary for progestational activity could be determined, it would then be possible to prepare nonsteroidal progestational compounds.

Djerassi *et al.* (1956c) determined that ring B of 8-isotestosterone is not of the chair form but of the boat form of steric configuration. If so, the stereochemical configuration of the molecule is not twisted and bent, but rather flat.

The reason that the 9-iso forms (9β,10α-pregn-4-ene-3,20-dione and 17α-hydroxy-9β,10α-pregn-4-ene-3,20-dione acetate) have increased activity is that the angular methyl group attached to carbon 10 is in the α position. Progestational activity is favored since the Δ^4 double bond is not sterically hindered in a close β position.

The fact that the 8-iso form (8α,9α-pregn-4-ene-3,20-dione) has decreased progestational activity shows that such a difference in stereochemical configuration is disadvantageous for a compound's having progestational activity.

It is interesting to notice that compounds having varying stereochemical configurations are progestationally active when given orally, whereas progesterone itself has little activity when given orally. Because of this phenomenon it is thought that there are two systems which play a role in the metabolism of progesterone, one which deactivates progesterone, and one which influences its physiological activity. The former is of greater importance in determining the active part of the structure of the steroids having progestational activity.

V. The Influence of 17α-Substitution in Testosterone and 19-Nortestosterone Derivatives

In general, those compounds which have a saturated or unsaturated alkyl group attached in position 17α of testosterone or 19-nortestosterone have high progestational activity. The relation between groups substituted in position 17α and their progestational activity is shown in Table VI.

The groups substituted at the 17α-position leading to compounds with high progestational activity may generally be divided into three categories: (A) groups in which the unsaturated conjugation is one carbon removed from C-17, such as 2-methallyl, allyl, 1-methallyl, and 2-butenyl (Fig. 13A); (B) saturated alkyl groups, such as isopropyl,

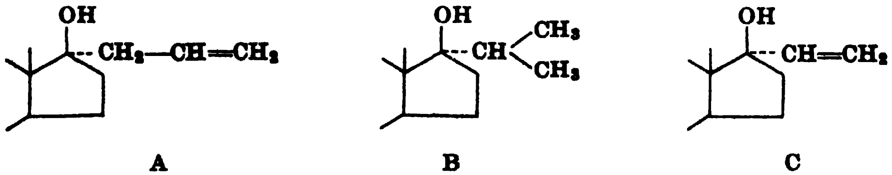
TABLE VI

THE PROGESTATIONAL ACTIVITY OF 17α-SUBSTITUTED 19-NORTESTOSTERONE DERIVATIVES

Compound	17α-Substituent	Progestational activity: Clauberg[a] (s.c.)	Clauberg[b] (oral)	McGinty[a] (intrauterine)
Estr-4-en-3-one, 17β-hydroxy-	H	0.05	0.2	0.005
Estr-4-en-3-one, 17β-hydroxy-17α-methyl-	Methyl	5	1	0.005
Estr-4-en-3-one, 17α-ethyl-17β-hydroxy-	Ethyl	5	2.5	0.25
Estr-4-en-3-one, 17β-hydroxy-17α-vinyl-	Vinyl	5	2.5	0.005
Estr-4-en-3-one, 17α-ethynyl-17β-hydroxy-	Ethynyl	0.5	1	0.005
Estr-4-en-3-one, 17β-hydroxy-17α-propyl-	Propyl	2.5	0.25	5
Estr-4-en-3-one, 17β-hydroxy-17α-isopropyl-	Isopropyl	20	—	—
Estr-4-en-3-one, 17β-hydroxy-17α-(2-propenyl)-	Allyl	10	2.5	0.5
Estr-4-en-3-one, 17β-hydroxy-17α-propynyl-	Propynyl	5	—	—
Estr-4-en-3-one, 17α-butyl-17β-hydroxy-	Butyl	1	<0.25	10
Estr-4-ene-3β, 17β-diol, 17α-(2-butenyl)-	Butenyl	2.5	—	10
Estr-4-en-3-one, 17β-hydroxy-17α-(1-methyl-2-propenyl)-	1-Methallyl	5	0.5	10
Estr-4-en-3-one, 17β-hydroxy-17α-(2-methallyl)-	2-Methallyl	25	10	10
Estr-4-en-3-one, 17β-hydroxy-17α-octyl-	Octyl	0.05	—	0.5–1

[a] Progesterone = 1.

[b] Subcutaneously injected progesterone = 1.



A B C

FIG. 13. Exemplification of the three categories of 17α-substituents that show high progestational activity.

methyl, ethyl, propyl, and butyl (Fig. 13B); (C) groups in which the unsaturated conjugation is directly associated with C-17, such as vinyl, 1-propynyl, and ethynyl (Fig. 13C).

It will be noticed in Fig. 14 that the electron density of the oxygen atom of the 17β-hydroxy group of the compounds of categories A and

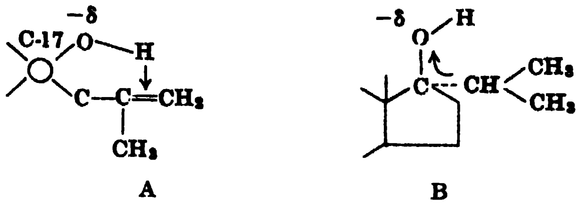


FIG. 14. The influence of the 17α-substituents on the electron density of the 17β-hydroxy group and their subsequent facilitation of acetylation of the hydroxyl group.

B should be high, being affected either by the hydrogen atom in the 17β-hydroxy group in relation to the double bond in the side chain of the compounds of category A (Fig. 14A) or by the I-effect of the substituted alkyl group of the compounds in category B (Fig. 14B). One might assume that the increase in electron density of the oxygen atom of the 17β-hydroxy group facilitates the acetylation of the hydroxyl group.

The condition existing in category C is quite different from that of categories A and B. In Table IV it is worth noting that the compounds of category C have little progestational activity when administered locally. It is therefore thought that in the case of subcutaneous or oral administration the unsaturated positions become saturated, thus enabling the compounds to show progestational activity.

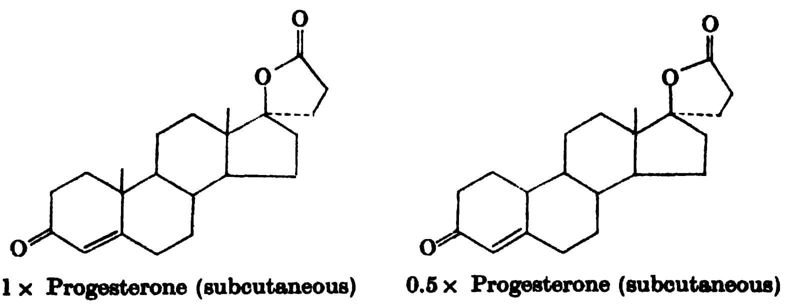


FIG. 15. Spirolactone forms of progesterone showing progestational activity.

Hertz and Tullner (1958) have reported several spirolactone forms of progesterone to have progestational activity. These forms together with their progestational activity are shown in Fig. 15. It is not possible for us to explain by our theory why these spirolactone forms elicit progestational activity. Possibly the activity appears as a result of the opening of the lactone ring.

VI. The Influence upon Progestational Activity of the Substitutional Site on the Steroid Nucleus

A. Substitution at Position 1

1α-Methyl-19-norprogesterone (1α-methyl-19-norpregn-4-ene-3,20-dione) and 1α-methyl-17α-ethynyl-19-nortestosterone (17α-ethynyl-17β-hydroxy-1α-methylestr-4-en-3-one) are progestationally approximately as active as progesterone. 1β-Methyl-19-norprogesterone is inactive, indicating that substitution in position 1β influences the character of the Δ^4 double bond.

B. Substitution at Position 2

16α-Methylprogesterone is as active as progesterone, whereas 2α,16α-dimethylprogesterone is inactive. 2α-Methyl- and 2β-methylprogesterone are also inactive. Substitution in position 2 therefore causes the disappearance of progestational activity. To date there are no reports concerning additional compounds substituted at C-2.

C. Substitution at Position 3

Until recently it was believed that oxygen at C-3, either as the 3β-hydroxy or 3-ketone group, was necessary for progestational activity. De Winter *et al.* (1959), however, found the 3-deoxy forms (17-alkylated 3-deoxo-19-nortestosterones, e.g., 17α-ethynylestr-4-en-17β-ol) to have high activity. Progestational activity has also been found for 3β-chloro-6-methyl-16α,17α-isopropylidenedioxypregn-5-en-20-one, a steroid with chlorine at C-3. There appears to be no relationship between the 3-hydroxy and 3-ketone groups and progestational activity.

D. Substitution at Position 4

4-Methylprogesterone (4-methylpregn-4-ene-3,20-dione) has 0.5 times the progestational activity of progesterone. When a hydroxy group is introduced at C-4 in the 17α-acetoxyprogesterone molecule, the resulting steroid, 4,17α-dihydroxypregn-4-ene-3,20-dione 17-acetate, has increased oral activity but decreased activity on subcutaneous administration. Although the 4-hydroxy group is not directly related to progestational activity per se, it does influence other determining factors.

In general it may be said that steroids with C-4 substituents show increased activity when administered orally and decreased activity when administered subcutaneously.

E. Substitution at Position 6

Compounds substituted at C-6β generally have decreased progestational activity, whereas compounds substituted in position 6α have increased activity, especially when administered by the oral route. This phenomenon was clearly demonstrated by Kincl (1961), who studied the 6α- and 6β-substituted forms of 17α-acetoxyprogesterone. He found that the 6α-substituted compounds were definitely more active than the 6β-substituted compounds when administered orally (Table VII).

TABLE VII

The Relative Progestational Activity of 6α- and 6β-Substituted Forms of 17α-Acetoxyprogesterone

Substituent at position 6 (molecular wt.)	Ratio: activity[a] of α-form/activity of β-form
Cl (35.46)	ca. 4–5
Br (79.92)	ca. 2
Br Δ^1	ca. 4–8
F (19.00)	ca. 10

[a] Oral Clauberg assay.

David *et al.* (1957) noted similar differences in activity with respect to 6α- and 6β-methylethisterone.

The above phenomenon cannot be explained by means of stereochemical hindrance, for the α/β ratio of progestational activity decreases with the increasing molecular weight of the substituent, as shown in Table VII.

In general, compounds having 6α-substituents elicit increased progestational activity when given orally. Increasing the molecular weight of the 6α-substituent, however, results in reduced activity.

F. Substitution at Position 7

7α-Acetylthio-17α-hydroxypregn-4-ene-3,20-dione propionate is as active a progestational agent as progesterone. It is thought that substitutional groups such as the acetylthio group are axial in the α-position and are removed easily. For this reason it is not clear what influence position 7α has on progestational activity. Additional studies on 7-substituted compounds for progestational activity are required.

G. Substitution at Positions 9 and 11

Compounds substituted in position 11 generally have lower activity than the parent compounds, but steroids substituted in both the 9α and 11β positions have increased oral progestational activity (Table VIII).

TABLE VIII

The Progestational Activity of 9- and 11-Substituted Derivatives of Progesterone

Compound	Progestational activity	
	Clauberg[a] (s.c.)	Clauberg[b] (oral)
Pregn-4-ene-3,20-dione, 9α-bromo-11β-chloro-	1	—
Pregn-4-ene-3,20-dione, 9α-bromo-11β-fluoro-	0.7	<1 (P)
Pregn-4-ene-3,20-dione, 9α-bromo-11β-hydroxy-	0.5	2–3 (ET)
Pregn-4-ene-3,20-dione, 9α,11β-dichloro-	5.5	—
Pregn-4-ene-3,20-dione, 9α-chloro-11β-fluoro-	—	1 (P)
Pregn-4-ene-3,20-dione, 9α-chloro-11β-hydroxy-	0.25	1 (ET)
Pregn-4-ene-3,20-dione, 9α-fluoro-11β-hydroxy-	0.125	3–5 (ET)
Pregn-4-ene-3,11,20-trione, 9α-bromo-	0.5	3–5 (ET)
Pregn-4-ene-3,11,20-trione, 9α-chloro-	0.25	1 (ET)
Pregn-4-ene-3,11,20-trione, 9α-fluoro-	0.25	2–3 (ET)

[a] Progesterone = 1.
[b] Subcutaneously injected progesterone = 1 where indicated by (P), ethisterone = 1 where indicated by (ET).

Few steroids with C-9 substituents only are known. 9-Hydroxyprogesterone was inactive in the McGinty test. 11β-Hydroxyprogesterone was inactive when administered subcutaneously (Table IX).

H. Substitution at Position 12

Although 12α-hydroxyprogesterone (12α-hydroxypregn-4-ene-3,20-dione) has weak progestational activity when administered subcutaneously (0.06 × progesterone), 11β-hydroxyprogesterone has increased progestational activity both orally and subcutaneously when a halogen is substituted in position 12 (Table IX). The substituted halogens are associated with increasing activity in the following order: F < Cl < Br.

TABLE IX

THE PROGESTATIONAL ACTIVITY OF 12α-HALOGENATED 11β-HYDROXYPROGESTERONE

Compound	Progestational activity		
	12α-Substituent	Clauberg[a] (s. c.)	Clauberg[b] (oral)
Pregn-4-ene-3,20-dione, 11β-hydroxy-	H	Inactive	—
Pregn-4-ene-3,20-dione, 12α-bromo-11β-hydroxy-	Br	2	2–3
Pregn-4-ene-3,20-dione, 12α-chloro-11β-hydroxy-	Cl	2	1
Pregn-4-ene-3,20-dione, 12α-fluoro-11β-hydroxy-	Fl	1	0.2

[a] Progesterone = 1.
[b] Ethisterone = 1.

I. Substitution at Positions 14 and 15

14-Hydroxy- and 15-hydroxyprogesterone are inactive in the McGinty test. Additional compounds having substituents in positions 14 or 15 have been reported in patents; however, their relative progestational activity is obscure. These compounds are the following: pregn-4-ene-3,11,15,20-tetraone; pregn-4-ene-3,11,20-trione, 14α-methyl-; pregn-4-ene-3,11,15-trione; pregn-4-ene-3,20-dione, 11β-hydroxy-14α-methyl-; pregn-4-ene-3,20-dione, 11β,15α-dihydroxy-; pregn-4-ene-3,20-dione, 11α,15β-dimethyl-.

J. Substitution at Position 16

16α-Methylprogesterone (16α-methylpregn-4-ene-3,20-dione) has the same degree of progestational activity as progesterone. Addition of a 16α-methyl group to the progestationally inactive 17α-hydroxyprogesterone failed to yield a progestationally active compound.

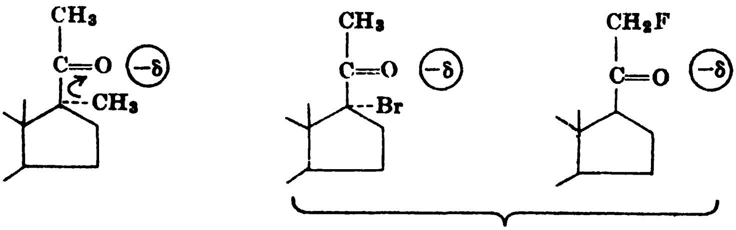
K. Substitution at Position 17

17α-Hydroxyprogesterone (17α-hydroxypregn-4-ene-3,20-dione) is not a progestationally active steroid. However, esterification of the 17α-hydroxy group with a fatty acid results in an orally active compound. These esters show prolonged progestational activity when administered subcutaneously.

L. Substitution at Position 20

Conversion of progesterone to 20-thioxypregn-4-en-3-one resulted in a compound less active than progesterone. Reduction of the 20-oxo group yielded 20α-hydroxypregn-4-en-3-one with an activity of 0.2–0.3 that of progesterone, and 20β-hydroxypregn-4-en-3-one which has 0.1–0.2 the activity of the parent compound. Both compounds were administered subcutaneously.

The 20-oxo group in progesterone produces a state of high electrical density at C-20 which appears to be associated with the progestational activity of progesterone. Electrical density at position 20 and progestational activity may be increased by means of substitution at position 17α with substituents such as a methyl group or bromine atom, or by substitution of fluorine at C-21. The method by which this may occur is illustrated in Fig. 16.



I-effect M-effect

FIG. 16. Methods by which the electrical density of position 20 may be increased.

M. Substitution at Position 21

As the molecular weight of the 21-substituents increases, progestational activity decreases (Section IV, C; Table IV). 21-Hydroxyprogesterone (21-hydroxypregn-4-ene-3,20-dione, deoxycorticosterone), however, is a very weak progestational agent in spite of the fact that the hydroxy group has a low molecular weight. This is due to the stereochemical hindrance exerted by the hydroxy group and the chemical

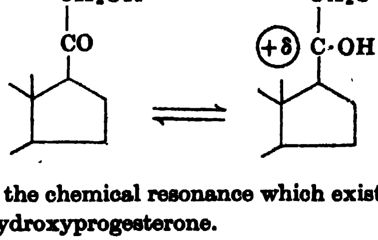


FIG. 17. Illustration of the chemical resonance which exists between the 21-hydroxy and 20-oxo groups of 21-hydroxyprogesterone.

resonance which exists between the 21-hydroxy and 20-oxo groups, causing decreased electrical density of the oxygen at position 20 (Fig. 17).

VII. The Influence of the Type of Substitutional Group on Progestational Activity

In the previous section it was demonstrated that an important factor enabling a steroid to have progestational activity is the position of the substitution. Also important is the type of group substituted at various positions, which will be considered in this section.

Alkyl or halogen substituents at position 6α yield steroids which elicit greater progestational activity than when the substitution is at position 6β. However, compounds having either 6α- or 6β-NO_2 groups substituted at C-6 have extremely low progestational activity. Bowers *et al.* (1959c) reported that 6α- and 6β-nitroprogesterone exhibited less than one-eighth the progestational activity of progesterone in the guinea pig copulatory assay. Addition of a 6α- or 6β-methyl group to the 16α,17α-isopropylidenedioxypregn-4-ene-3,20-dione molecule results in compounds having the same progestational activity as the parent compound. Addition of a 6β-fluoro group to the latter steroid, however, gives a compound which is inactive when administered subcutaneously.

16α-Methylprogesterone (16α-methylpregn-4-ene-3,20-dione) has approximately the same activity as progesterone, whereas 16α-hydroxyprogesterone is inactive. Drill and Riegel (1958) have reported that 16α-chloroprogesterone has low activity, whereas 16α-bromoprogesterone has moderate activity.

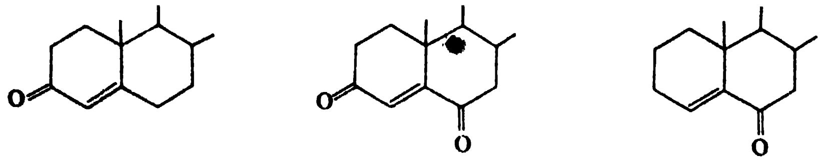
Another example of the influence of substitutional groups on progestational activity is that of the different halogen combinations of 9α,11β-dihalopregn-4-ene-3,20-dione. Table VIII shows the variations in progestational activity caused by changing the halogen atoms at positions 9α and 11β. It will be noticed that when both substituents are chlorine atoms, the compound has the greatest activity.

6α-Nitropregn-4-ene-3,20-dione   Pregn-4-ene-3,6,20-trione

FIG. 18. The possible relation between the Δ^4 double bond and the high electrica density at position 6 when an oxo or NO_2 group is substituted at position 6.

6-Ketoprogesterone (pregn-4-ene-3,6,20-trione) is inactive, perhaps owing to a high negative electrical density at C-6. This may also be the reason for the inactivity of 6α-nitroprogesterone (Fig. 18).

Figure 19 shows the three combinations possible when a ketone group is substituted in positions 3 and (or) 6. That shown in Fig. 19A is progestationally active, that of Fig. 19B is inactive. It is expected that the Δ^4-6-keto-3-deoxy structure (Fig. 19C) may be compatible with progestational activity.

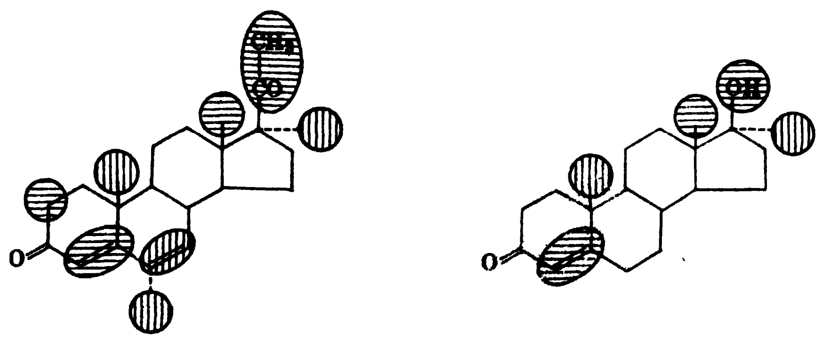


A B

FIG. 19. Possible combinations when substituting a ketone group in positions 3 and (or) 6.

VIII. The Minimum Structural Components Necessary for the Appearance of Progestational Activity

It was mentioned previously that the minimum structural components necessary for progesterone to elicit progestational activity are an unsubstituted position 2, a Δ^4 double bond, a 17β-acetyl group, and an 18β-methyl group. Substituents at positions 6α, 17α, and 19 on the steroid nucleus of progesterone and the presence of a Δ^6 double bond increase progestational activity (Fig. 20A).



A
Progesterone

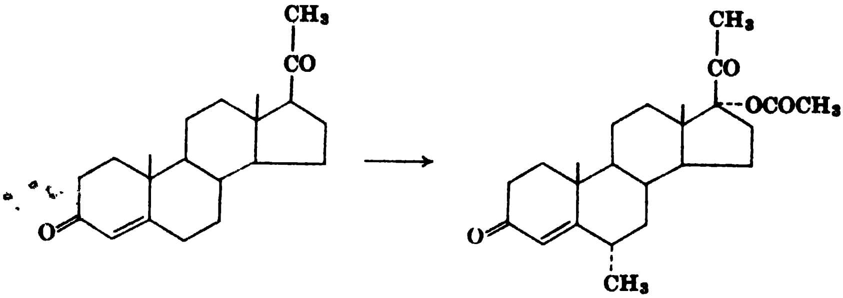
B
Testosterone

FIG. 20. Structural requirements necessary for the appearance of, and increases in, progestational activity. The minimum necessary structural components for the appearance of progestational activity are indicated in horizontal lines. Those positions that are important for the increasing progestational activity are indicated in vertical lines.

The minimum structural components necessary for testosterone to elicit progestational activity are a Δ^4 double bond, a 17β-hydroxy group, and an 18β-methyl group. Positions 17α and 19 on the steroid nucleus of testosterone play an important role in increasing the progestational activity (Fig. 20B).

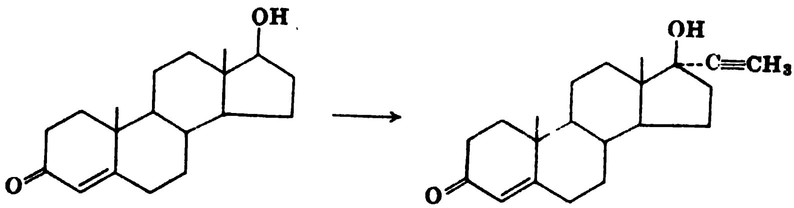
IX. Potent Progestational Steroids

Highly active progestational steroids are derived from progesterone, testosterone, and 19-nortestosterone (Fig. 21). Recently, derivatives of each of these three compounds have been synthesized which have considerable progestational activity (Fig. 21).



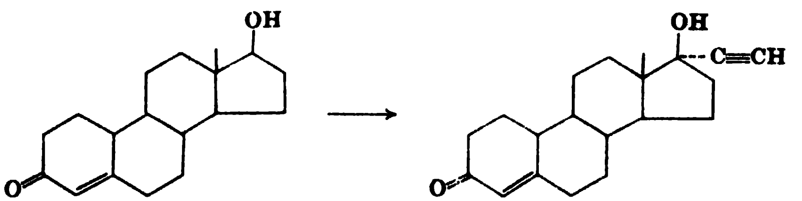
Progesterone

6α-Methyl-17α-hydroxypregn-4-ene-3,20-dione acetate



Testosterone

17α-Ethynyl-17β-hydroxyandrost-4-en-3-one (Ethisterone)



19-Nortestosterone

17α-Ethynyl-17β-hydroxyestr-4-en-3-one (Norethisterone)

FIG. 21. Derivatives of progesterone, testosterone, and 19-nortestosterone which have high progestational activity.

Progesterone derivatives having high progestational activity are listed in Table X. It should be noted that although these compounds are highly active when administered orally, only several, namely, 19-

TABLE X

PROGESTERONE DERIVATIVES HAVING HIGH ACTIVITY IN THE ORAL CLAUBERG ASSAY

Compound	Progestational activity[a]
Pregna-1,4-diene-3,20-dione, 6α-bromo-17α-hydroxy-, acetate	6× ENT
Pregna-1,4-diene-3,20-dione, 6α-chloro-17α-hydroxy-, acetate	8× ENT
Pregna-1,4-diene-3,20-dione, 6α-fluoro-17α-hydroxy-, acetate	6× ENT
Pregna-1,4-diene-3,20-dione, 17α-hydroxy-6α-methyl-, acetate	8× ENT
Pregna-1,4,6-triene-3,20-dione, 6-chloro-17α-hydroxy-, acetate	35× ENT
Pregna-1,4,6-triene-3,20-dione, 6-fluoro-17α-hydroxy-, acetate	8× ENT
Pregna-1,4,6-triene-3,20-dione, 17α-hydroxy-6-methyl-, acetate	10× ENT
Pregna-1,4,6-triene-3,20-dione, 17α-hydroxy-16α-methyl-, acetate	66× AP
Pregn-4-ene-3,20-dione, 17α-ethyl-6α-methyl-	5× ENT
Pregn-4-ene-3,20-dione, 9α-fluoro-11β,17α-dihydroxy-, 17-acetate	25× P
Pregn-4-ene-3,20-dione, 17α-hydroxy-6α-methyl-, acetate	10× ENT
Pregn-4-ene-3,20-dione, 6α-methyl-17α-hydroxy-, acetate	60–75× AP
Pregna-4,6-diene-3,20-dione, 6-chloro-17α-ethyl-	20× ENT
Pregna-4,6-diene-3,20-dione, 6-chloro-21-fluoro-17α-methyl-	10× ENT
Pregna-4,6-diene-3,20-dione, 6-chloro-17α-hydroxy-, acetate	35–50× ENT
Pregna-4,6-diene-3,20-dione, 6-chloro-17α-methyl-	20× ENT
Pregna-4,6-diene-3,20-dione, 17α-ethyl-6-methyl-	20× ENT
Pregna-4,6-diene-3,20-dione, 6-fluoro-17α-hydroxy-, acetate	15× ENT
Pregna-4,6-diene-3,20-dione, 21-fluoro-17α-hydroxy-6-methyl-, acetate	17× P
Pregna-4,6-diene-3,20-dione, 17α-hydroxy-6-methyl-, acetate	12× ENT
Pregna-4,6-diene-3,20-dione, 17α-hydroxy-16α-methyl-, acetate	15× AP
Pregna-4,6-diene-3,20-dione, 6,17α-dimethyl-	10× ENT

[a] AP: Compared to 17α-acetoxyprogesterone (oral), which equals 1. ENT: Compared to norethisterone (oral), which equals 1. P: Compared to subcutaneously administered progesterone, which equals 1.

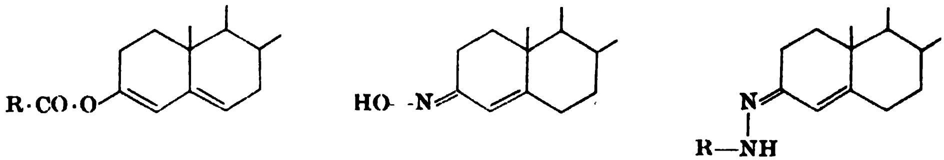
norprogesterone, retroprogesterone, and 17α-alkoxyprogesterone, have increased activity when administered subcutaneously. With the exception of the 19-nor derivatives, administration of the compounds in depot form results in increased activity which may be due to retardation of inactivation.

X. Progestational Compounds Having Prolonged Activity

Compounds known to have prolonged progestational activity may be classified chemically into three groups: (A) compounds substituted at position 3; (B) those substituted at position 17α; and (C) those substituted at positions 16α and 17α.

A. Compounds Substituted at Position 3

Compounds substituted at position 3 may be divided into the following three subgroups: (1) enol ester, (2) oxime, and (3) hydrazone (Fig. 22).



Enol ester Oxime Hydrazone

FIG. 22. Position 3 substituents.

Various fatty acid enol esters were studied by Junkmann (1954), who noted that the substitution of a trimethylacetate or benzoate group resulted in increased and prolonged progestational activity. Other esters, however, had approximately the same activity as progesterone.

3-Hydroxyiminopregn-4-en-20-one, which has an oxime substituted for the 3-keto group, is about as active as progesterone, whereas 3-*N*-phenyliminopregn-4-en-20-one, which has a phenylimino group substituted in position 3, is probably more active than progesterone, although the activity was not prolonged.

Gleason and Parker (1959) reported that the hydrazone derivative of 17α-heptanonyloxyprogesterone (3-benzilovlhydrazone-17α-hydroxypregn-4-en-20-one enanthate) (Fig. 23) has more prolonged activity than 17α-hydroxyprogesterone enanthate.

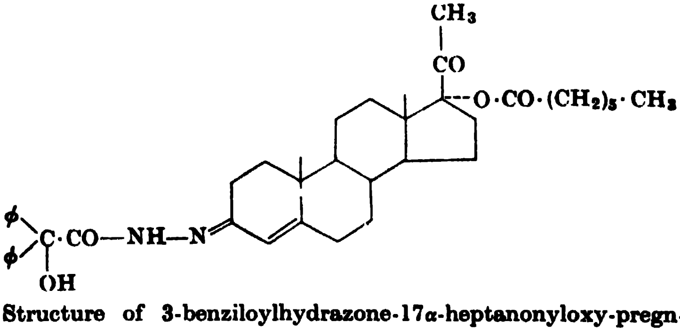


FIG. 23. Structure of 3-benziloylhydrazone-17α-heptanonyloxy-pregn-4-en-20-on

B. Compounds Substituted at Position 17α

17α-Hydroxyprogesterone, having a hydroxy group substituted at position 17α, is less than 0.01 as active as progesterone. Esterification of this hydroxy group, however, results in increased progestational activity when administered both orally and subcutaneously. Junkmann (1954) reported that the acetate, butyrate, and caproate forms had both increased and prolonged activity, that of the caproate form being most striking. Increasing the length of the carbon chain of the ester results in increased activity; however, the 11-carbon undecylate ester elicits decreased activity.

Since Junkmann's report, the prolonged activity of 17α-hydroxyprogesterone caproate has been studied by Kessler and Borman (1957) and confirmed by Diczfalusy (1960). Kessler and Borman found that when single doses were administered, 17α-hydroxyprogesterone caproate had 5 times more prolonged activity than progesterone. It has been established, therefore, that the substitution of a fatty acid ester containing 6–8 carbons for the 17α-hydroxy group results in increased and prolonged progestational activity.

Diczfalusy (1960) reported that the substitution of a 3-(*p*-butoxyphenyl)-propyloxy group in position 17α of progesterone (Fig. 24) yields a compound having increased and prolonged progestational activity similar to that of 17α-hydroxyprogesterone caproate. Increased and prolonged progestational activity is not entirely due, therefore, to the length of the substituted groups.

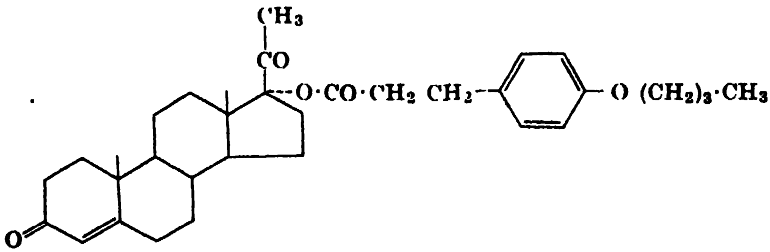


FIG. 24. Structure of 17α-[3-(*p*-butoxyphenyl)-propyloxy]-pregn-4-ene-3,20-dione.

It was mentioned above that 3-benziloylhydrazone-17α-hydroxypregn-4-en-20-one enanthate had increased and prolonged progestational activity. The 3-enolacetate form of 17α-acetoxyprogesterone (Fig. 25), however, has decreased activity (Junkmann, 1954).

C. Compounds Substituted at Positions 16α and 17α

As in the case of 17α-hydroxyprogesterone, 16α,17α-dihydroxyprogesterone (16α,17α-dihydroxypregn-4-ene-3,20-dione) has almost

no progestational activity. Compounds ketalized by acetone (Fig. 26A) or by acetophenone (Fig. 26B) (Lerner *et al.*, 1961a) and also 16α,17α-epoxypregn-4-ene-3,20-dione (Fig. 26C) (Junkmann, 1954), which has positions 16 and 17 linked to a single oxygen, have progestational

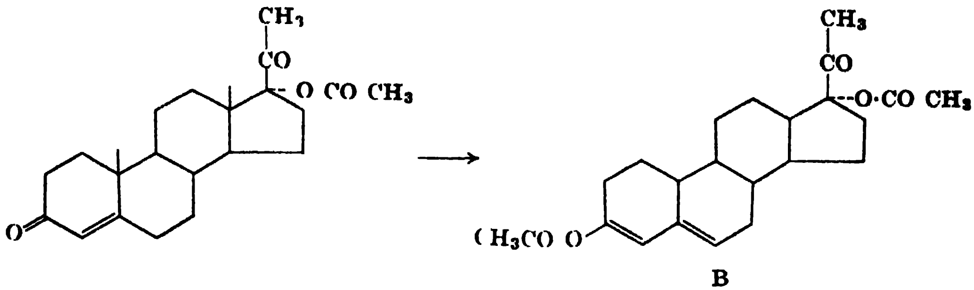


FIG 25 The structure of the 3 enolacetate form of 17α acetoxyprogesterone (3,17α dihydroxypregna 3,5 dien 20 one diacetate)

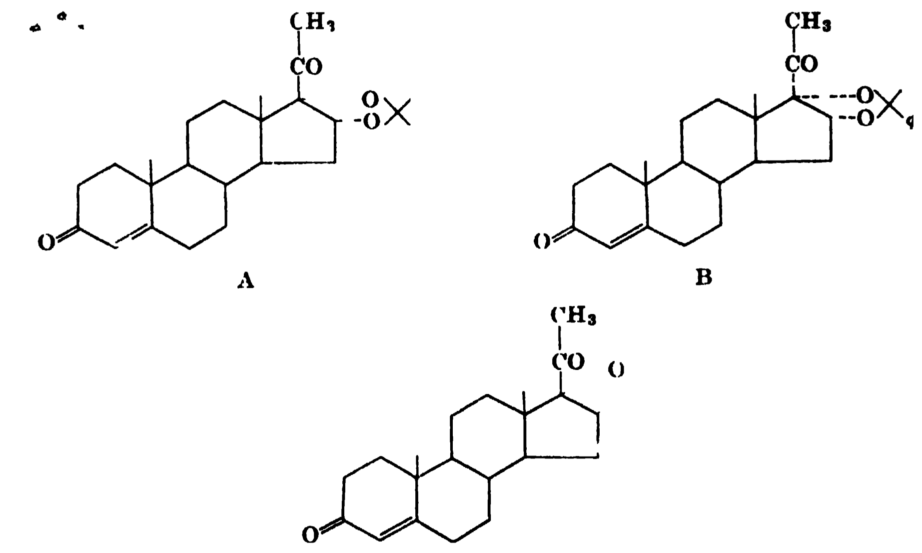


FIG 26 Structures of several 16α,17α substituted forms of progesterone

activity. The epoxy derivative (Fig. 26C) has approximately 0.5 the progestational activity of progesterone, whereas the ketal forms of progesterone shown in Figs. 26A and B when administered orally have greater and more prolonged activity than progesterone administered subcutaneously. When administered subcutaneously, the 2-acetofuran derivative is 32–64 times more active than progesterone. Figure 27 shows

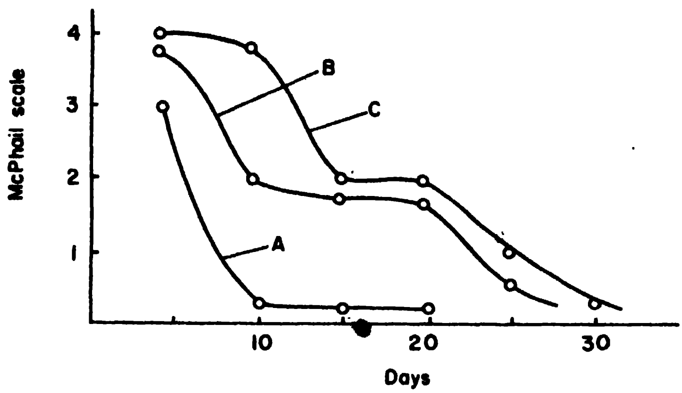


FIG. 27. Prolonged progestational activity (McPhail scale) elicited by ketal forms of progesterone (from Lerner *et al.*, 1961a). A: Progesterone; B: Pregn-4-ene-3,20-dione, 16α,17α-acetone ketal; C: Pregn-4-ene-3,20-dione,16α,17α-acetophenone ketal.

the prolongation of progestational activity resulting from a single 10-mg dose of each of the two ketal forms.

The fact that the enol ester attached to C-3 of progesterone causes prolonged activity (reported by Diczfalusy, 1960) is thought to be due to the same phenomenon as that concerned with the substitution of fatty acid esters in position 17 of testosterone and estradiol-17β. Although it is necessary that testosterone and estradiol-17β have a 17β-hydroxy group in order for them to elicit their respective activities, it is not necessary for progesterone to have either a 3-hydroxy or a 3-keto group to produce progestational activity (Section VI, C). In the case of the esterification of testerone and estradiol-17β, it is unclear whether the prolonged activity is due to hydrolysis of the ester or due to activity inherent in the ester. Likewise, with respect to progesterone it is not clear whether the prolonged activity due to esterification is related to changes in its uptake, its metabolism, or its inactivation.

17α-Hydroxyprogesterone and 16α,17α-dihydroxyprogesterone, which should be produced by the hydrolysis of the corresponding ester or ketal are not progestationally active, whereas the combined forms are active. It is therefore thought that the activity is due to the ester or ketal forms themselves or to some other metabolite.

It was indicated in Section III that the introduction of the 17α-hydroxy group produces an inactive compound due to the change in the stereochemical direction of the 17β-acetyl radical. The 17α-alkoxy and 16α,17α-ketal forms of progesterone do not themselves influence the activity of progesterone, and therefore removal of the free 17α-hydroxy group restores the progestational activity. In addition, introduction of

such substituted forms results in a decreased rate of metabolism, which in turn causes increased and prolonged activity.

In general, those compounds that have prolonged progestational activity also have high progestational activity, the peak of the activity after the time of injection being delayed in comparison with that of progesterone. For example, Kessler and Borman (1957) reported that the time curve for the progestational activity of a single injection of 20 mg of 17α-hexanoyloxyprogesterone compared with that of a 20-mg injection of progesterone is that shown in Fig. 28. The greater and more

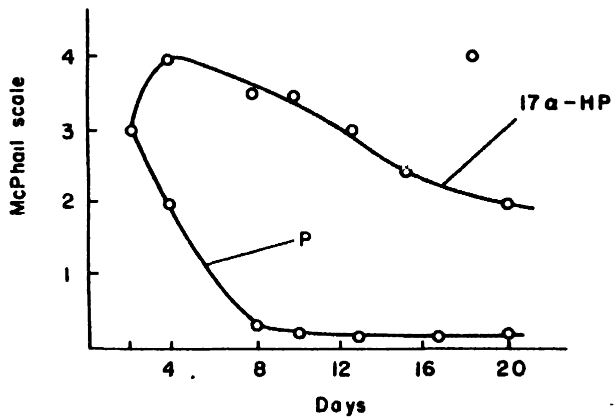


FIG. 28. The prolonged progestational activity (McPhail scale) of 17α-hexanoyloxyprogesterone. P = Progesterone; 17α-HP = 17α-hexanoyloxyprogesterone.

prolonged activity may be explained in two ways. The first is that the compound may be metabolized to an active form. Secondly, the compound may be absorbed slowly, therefore reaching the uterine endometrium slowly. In addition, it is thought that the active compound itself may accumulate. In the case of highly active agents, it is not certain whether the activity is due to the compound itself, or is due to an accumulation of highly active metabolites.

XI. Compilation of Steroids Tested for Progestational Activity

A brief summary in tabular form of the progestational activity of approximately 450 steroids gleaned from the literature is given in Table XI. Each compound is listed alphabetically according to its systematic name. Substituents attached to the basic steroid molecule are arranged alphabetically following the root name.

TABLE XI

COMPILATION OF STEROIDS TESTED FOR PROGESTATIONAL ACTIVITY[a]

Compound	Animal	Test	Route	Response	Reference
5α-Androstan-17β-ol, 3,3′-azine-2α,17α-dimethyl-	Rbt	Cl.	SC	Inactive	Piotti *et al.* (1962)
	Rbt	Cl.	O	Inactive	Piotti *et al.* (1962)
Androsta-1,4-dien-3-one, 17α-ethynyl-6α-fluoro-17β-hydroxy-	Rbt	Cl.	O	0.5 × ENT	Knox *et al.* (1960)
Androsta-1,4-dien-3-one, 17α-ethynyl-17β-hydroxy-	Rbt	Cl.	O	Sl. < ET	Ringold *et al.* (1959b)
Androsta-1,4,6-trien-3-one, 17α-ethynyl-9α-fluoro-11β,17β-dihydroxy-	—	—	O	Active (> non-halo. parent)	Gould *et al.* (1957)
Androsta-1,4,6-triene-3,11-dione, 17α-ethynyl-9α-fluoro-17β-hydroxy-	—	—	—	Active (> non-halo. parent)	Gould *et al.* (1957)
Androst-4-ene-3β,17β-diol, 17α-ethynyl-	Rbt	Cl.	O	1 × ET	Sondheimer and Klibansky (1959)
Androst-4-en-3-one, 17α-(1-butynyl)-17β-hydroxy-	Rbt	Cl.	O	1.6 × ET	David *et al.* (1957)
	Rbt	Cl.	O	> ET	Barton *et al.* (1959)
Androst-4-en-3-one, 17α-(1-butynyl)-17β-hydroxy-6α-methyl-	Rbt	Cl.	O	9.2 × ET	David *et al.* (1957)
Androst-4-en-3-one, 17α-(β-carboxyethyl)-17β-hydroxy-, γ-lactone	Rbt	Cl.	SC	0.1 × P	Hertz and Tullner (1958)
Androst-4-en-3-one, 6α-chloro-17α-ethynyl-17β-hydroxy-	Rbt	Cl.	O	1 × ENT	Knox *et al.* (1960)
Androst-4-en-3-one, 6α-chloro-17α-ethynyl-17β-hydroxy-, acetate	Rbt	Cl.	O	0.5 × ENT	Knox *et al.* (1960)
Androst-4-en-3-one, 6β-ethyl-17α-ethynyl-17β-hydroxy-	Rbt	Cl.	O	Inactive	David *et al.* (1957)
Androst-4-en-3-one, 17α-ethynyl-6α-fluoro-17β-hydroxy-	Rbt	Cl.	O	1 × ENT	Knox *et al.* (1960)
Androst-4-en-3-one, 17α-ethynyl-6α-fluoro-17β-hydroxy-, acetate	Rbt	Cl.	O	< 0.2 × ENT	Knox *et al.* (1960)

Androst-4-en-3-one, 17α-ethynyl-17β-hydroxy-	Rbt	Cl.	SC	0.3 × P	Meystre *et al.* (1948)
	Rbt	C-A	SC	0.3 × P	Meystre *et al.* (1948)
	Rbt	Cl.	O	±	Andreoli (1962)
	—	Decid.	O	Inactive	Andreoli (1962)
	—	P-M	O	Inactive	Andreoli (1962)
	Rbt	Cl.	O	0.06 × ENT	Madjerek *et al.* (1960)
	M	Decid.	—	Inactive	Madjerek *et al.* (1960)
	R	P-M	—	Inactive	Madjerek *et al.* (1960)
	R	P-M	SC	Inactive	Stucki (1958)
	M	H-F	IU	0.0012 × P	Zarrow *et al.* (1957)
	M	H-F	IU	Inactive	Nakao *et al.* (1958)
	Mky	EIWB	O	Inactive	Tullner and Hertz (1957)
Androst-4-en-3-one, 17α-ethynyl-17β-hydroxy-, acetate	Rbt	Cl.	SC	< P	Junkmann (1954)
Androst-4-en-3-one, 17α-ethynyl-17β-hydroxy-, butyrate	Rbt	Cl.	SC	> P	Junkmann (1954)
Androst-4-en-3-one, 17α-ethynyl-17β-hydroxy-, enanthate	Rbt	Cl.	SC	> P	Junkmann (1954)

continued

[a] In order to make the table as compact as possible, a number of items have been given coded abbreviations. These abbreviations are defined below according to the table heading under which they appear.

Animal: M = mouse; Mky = monkey; R = rat; Rbt = rabbit.

Test system: C-A = Corner-Allen; Cl. = Clauberg (including modifications of the basic Clauberg test); Carb. An. = carbonic anhydrase; Decid. = Deciduoma tests; EIWB = estrogen-induced withdrawal bleeding; GMR = glandular mucosal ratio; H-F = Hooker-Forbes; McG. = McGinty intrauterine test; P-M = pregnancy maintenance test.

Route: IM = intramuscular injection; IU = intrauterine injection; O = administered orally; SC = subcutaneous injection.

Response: AP = 17α-acetoxyprogesterone; DAP = 17α,21-diacetoxyprogesterone; ENT = 17α-ethynyl-19-nortestosterone (norethisterone); ET = 17α-ethynyltestosterone (ethisterone); NP = 19-norprogesterone; NT = 19-nortestosterone; P = progesterone; Sl = slight or slightly.

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Androst-4-en-3-one, 17α-ethynyl-17β-hydroxy-6α-methyl-	Rbt	Cl.	O	6.5 × ET	David *et al.* (1957)
	—	—	O	2 × ET	Campbell *et al.* (1958)
	Rbt	Cl.	—	6 × ET	Barton *et al.* (1959)
Androst-4-en-3-one, 17α-ethynyl-17β-hydroxy-6β-methyl-	—	—	O	1 × ET	Campbell *et al.* (1958)
	Rbt	Cl.	O	0.33 × ET	David *et al.* (1957)
Androst-4-en-3-one, 17α-(1-hexynyl)-17β-hydroxy-	Rbt	Cl.	O	0.5 × ET	David *et al.* (1957)
Androst-4-en-3-one, 17α-hydroxy-	Rbt	McG.	IU	Low activity	Salhanick and Swanson (1960)
Androst-4-en-3-one, 17β-hydroxy-	Rbt	McG.	IU	Mod. activity (< P)	Salhanick and Swanson (1961)
	Rbt	McG.	IU	Highly active	Salhanick and Swanson (1960)
	M	H-F	IU	Inactive	Hooker and Forbes (1947)
Androst-4-en-3-one, 17β-hydroxy-, propionate	Rbt	Carb. An.	SC	Inactive	Lutwak-Mann (1955)
	Rbt	McG.	—	< 0.01 × P	Saunders *et al.* (1957a)
	M	H-F	IU	Active	Nakao *et al.* (1958)
Androst-4-en-3-one, 17β-hydroxy-6α-methyl-17α-(1-propynyl)-	Rbt	Cl.	O	11.5 × ET	David *et al.* (1957, 1959)
	Rbt	Cl.	O	0.25 × ENT	Madjerek *et al.* (1960)
	M	Decid.	—	Poor	Madjerek *et al.* (1960)
	R	P-M	—	Inactive	Madjerek *et al.* (1960)
	Rbt	Cl.	O	Active	Andreoli (1962)
	—	Decid.	O	±	Andreoli (1962)
	—	P-M	O	Inactive	Andreoli (1962)
	Rbt	Cl.	—	12 × ET	Barton *et al.* (1959), Hartley (1962)
	—	—	O	Potent	Ellis *et al.* (1960)
Androst-4-en-3-one, 17β-hydroxy-17α-methyl-	R	P-M	SC	Inactive	Stucki (1958)
Androst-4-en-3-one, 17β-hydroxy-17α-(1-pentynyl)-	Rbt	Cl.	O	1 × ET	David *et al.* (1957)
Androst-4-en-3-one, 17β-hydroxy-17α-propargyl-	—	—	—	Weak	Magrath *et al.* (1951)

Androst-4-en-3-one, 17β-hydroxy-17α-(1-propynyl)-	Rbt	Cl.	O	$3.0 \times$ ET	David *et al.* (1957)
	Rbt	Cl.	O	$>$ ET	Barton *et al.* (1959)
Androst-4-en-3-one-17-thione	Rbt	Cl.	SC	$< 0.05 \times$ P	Drill and Riegel (1958)
	Rbt	McG.	IU	$0.05 \times$ P	Drill and Riegel (1958)
Androst-4-ene-3,6,17-trione	M	H-F	IU	Active	Nakao *et al.* (1958)
Androst-4-ene-3,11,17-trione	M	H-F	IU	Active	Nakao *et al.* (1958)
Androst-4-ene-3,17-dione	Rbt	McG.	IU	Low activity	Salhanick and Swanson (1960)
Androsta-4,6-diene-3β,17β-diol, 17α-ethynyl-	Rbt	Cl.	O	Inactive	Baran (1963)
Androsta-4,6-diene-3β,17β-diol, 17α-ethynyl-, 3-acetate	Rbt	Cl.	O	$0.05 \times$ P (SC)	Baran (1963)
Androsta-4,6-diene-3β,17β-diol, 17α-ethynyl-, diacetate	Rbt	Cl.	O	Inactive	Baran (1963)
Androsta-4,6-diene-3β,17β-diol, 17α-ethynyl-, 17-acetate	Rbt	Cl.	O	$0.05 \times$ P (SC)	Baran (1963)
Androsta-4,6-diene-3β,17β-diol, 17α-ethynyl-, 3-propionate	Rbt	Cl.	O	$0.05 \times$ P (SC)	Baran (1963)
Androsta-4,6-dien-3-one, 6-chloro-17α-ethynyl-17β-hydroxy-	Rbt	Cl.	O	$0.3 \times$ ENT	Knox *et al.* (1960)
Androsta-4,6-dien-3-one, 17α-ethynyl-17β-hydroxy-	Rbt	Cl.	O	$0.05 \times$ P (SC)	Baran (1963)
Androsta-4,6-dien-3-one, 17α-ethynyl-17β-hydroxy-, acetate	Rbt	Cl.	O	$0.05 =$ P (SC)	Baran (1963)
Androsta-4,6-dien-3-one, 17α-ethynyl-17β-hydroxy-6-methyl-	—	—	O	$2 \times$ ET	Ellis *et al.* (1962)
Androsta-4,11-dien-3-one, 17α-ethynyl-17β-hydroxy-	Rbt	Cl.	SC	$<$ ET	Meystre and Wettstein (1949)
Androst-5-ene-3β,17β-diol, 17α-methyl-	M	H-F	IU	Inactive	Nakao *et al.* (1958)
Androst-5-ene-3β,17β-diol, 17α-propargyl-, 3-acetate	—	—	—	Weak activity	Magrath *et al.* (1951)
Androst-5-en-17-one, 3β-hydroxy-	M	H-F	IU	Active	Nakao *et al.* (1958)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Estran-3-one, 5β,10β-epoxy-17α-ethynyl-17β-hydroxy-	Rbt	Cl.	O	< 0.25 × ENT	Ruelas *et al.* (1958)
5α-Estran-3-one, 17α-ethyl-17β-hydroxy-	Rbt	Cl.	SC	0.5–1.0 × P	Edgren *et al.* (1959)
	Rbt	Cl.	SC	0.25 × P	Drill and Riegel (1958)
	Rbt	Cl.	O	0.1 × P (SC)	Drill and Riegel (1958)
	Rbt	McG.	IU	0.5 × P	Drill and Riegel (1958)
	Rbt	Cl.	O	0.01 × ENT	Kincl and Folch-Pi (1962a)
Estran-3-one, 17α-ethynyl-5α-fluoro-11β,17β-dihydroxy-	Rbt	Cl.	O	< 0.25 × ENT	Ruelas *et al.* (1958)
5α-Estran-3-one, 17α-ethynyl-17β-hydroxy-	Rbt	Cl.	SC	Inactive	Edgren *et al.* (1959)
5α-Estran-3-one, 17β-hydroxy-	Rbt	Cl.	SC	Inactive	Edgren *et al.* (1959)
5α-Estran-3-one, 17β-hydroxy-17α-methyl-	Rbt	Cl.	SC	0.1 × P	Edgren *et al.* (1959)
	Rbt	Cl.	SC	0.1 × P	Saunders (1958)
	—	—	IU	< 0.05 × P	Saunders (1958)
	Rbt	Cl.	SC	0.1 × P	Drill and Riegel (1958)
	Rbt	Cl.	O	< 0.1 × P (SC)	Drill and Riegel (1958)
	Rbt	McG.	IU	< 0.005 × P	Drill and Riegel (1958)
5α-Estran-3-one, 17β-hydroxy-17α-isopropyl-	Rbt	Cl.	SC	Active	Edgren *et al.* (1959)
5α-Estran-3-one, 17β-hydroxy-17α-n-propyl-	Rbt	Cl.	SC	Inactive	Edgren *et al.* (1959)
	Rbt	Cl.	SC	< 0.05 × P	Drill and Riegel (1958)
	Rbt	McG.	IU	< 0.01 × P	Saunders *et al.* (1957a)
5α,10α-Estran-3-one, 17β-hydroxy-17α-n-propyl-	Rbt	Cl.	SC	Inactive	Edgren *et al.* (1959)
5α-Estr-1-en-3-one, 17α-ethyl-17β-hydroxy-	—	—	—	Active (> 10-methyl deriv.)	Counsell (1961)
5α-Estr-1-en-3-one, 17β-hydroxy-17α-methyl-	—	—	—	Active (> 10 methyl deriv.)	Counsell (1961)
Estra-1,3,5(10)-triene-3,16α,17β-triol	M	H-F	IU	Active	Nakao *et al.* (1958)

Estra-1,3,5(10)-triene-3,17β-diol	M	H-F	IU[b]	Inactive	Hooker and Forbes (1947), Nakao *et al.* (1958)
Estra-1,3,5(10)-trien-17-one, 3-hydroxy-	M	H-F	IU	Inactive	Nakao *et al.* (1958), Hooker and Forbes (1947)
Estra-3,5-dien-17β-ol, 3-cyclopentoxy-17α-ethynyl-	Rbt	Cl.	—	1 × ENT	Falconi and Ercoli (1961)
	R	Inhib estrus	O	4 × ENT	Falconi and Ercoli (1961)
Estr-4-ene-3,17β-diol, 17α-ethynyl-	Rbt	Cl.	O	1 × ENT	Sondheimer and Klibansky (1959)
Estr-4-en-17β-ol, 17α-butyl-	—	—	—	Potent	de Winter *et al.* (1959)
Estr-4-en-17β-ol-, 17α-ethyl-	—	—	—	Potent	de Winter *et al.* (1959)
	Rbt	Cl.	O	2 × ENT	Kincl and Folch-Pi (1962a)
Estr-4-en-17β-ol, 16α-ethynyl-	Rbt	Cl.	O	1 5 × ENT	Kincl and Folch-Pi (1962a)
Estr-4-en-17β-ol, 17α-ethynyl-	—	—	—	Potent	de Winter *et al.* (1959)
	Rbt	Cl.	O	Active	Andreoli (1962)
	Rbt	Cl.	O	1 × P (SC)	Overbeek *et al.* (1962)
	Rbt	C-A	O	1 × P (SC)	Overbeek *et al.* (1962)
	M	Decid.	—	Inactive	Overbeek *et al.* (1962)
	—	Decid.	O	±	Andreoli (1962)
	—	P-M	O	Inactive	Andreoli (1962)
	R	P-M	—	Inactive	Overbeek *et al.* (1962)
Estr-4-en-17β-ol, 17α-methyl-	—	—	—	Potent	de Winter *et al.* (1959)
Estr-4-en-17β-ol, 17α-(2-propenyl)-	—	—	—	Potent	de Winter *et al.* (1959)
	Rbt	Cl.	O	0.5–0.8 × ENT	Andreoli (1962), Kincl and Folch-Pi (1962a), Madjerek *et al.* (1960)
	Rbt	Carb. An.	—	Active	Madjerek *et al.* (1960)
	Rbt	C-A	—	Active	Madjerek *et al.* (1960)
	M	Decid.	—	> ENT	Madjerek *et al.* (1960)
	—	Decid.	O	Active	Andreoli (1962)
	—	P-M	O	Active	Andreoli (1962)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Estr-4-en-17β-ol, 17α-(2-propenyl)-	R	P-M	SC	Active	Suchowsky (1962)
	R	P-M	O	Active	Suchwskoy (1962)
	R	P-M	—	>ENT	Madjerek *et al.* (1960)
Estr-4-en-17β-ol, 17α-propyl-	—	—	—	Potent	de Winter *et al.* (1959)
Estr-4-en-3-one, 17α-butenyl-17β-hydroxy-	Rbt	Cl.	SC	2.5 × P	Saunders *et al.* (1957a)
	Rbt	McG.	IU	10 × P	Saunders *et al.* (1957a)
	Rbt.	Carb. An.	SC	3.5 × P	Miyake and Pincus (1958)
	Rbt	Carb. An.	O	1.3 × AP	Miyake and Pincus (1958)
	Rbt	GMR	SC	2.4 × P	Miyake and Pincus (1958)
	Rbt	GMR	O	2.2 × AP	Miyake and Pincus (1958)
Estr-4-en-3-one, 17α-butyl-17β-hydroxy-	Rbt	McG.	IU	5 × P	Saunders *et al.* (1957a)
	Rbt	Cl.	SC	1 × P	Drill and Riegel (1958), Edgren *et al.* (1959)
	Rbt	Cl.	O	< 0.25 × P (SC)	Drill and Riegel (1958)
	Rbt	McG.	IU	10 × P	Drill and Riegel (1958)
	R	P-M	SC	Inactive	Saunders and Elton (1959)
Estr-4-en-3-one, 17α-(β-carboxyethyl)-17β-	Rbt	Cl.	SC	0.5 × P	Hertz and Tullner (1958)
hydroxy-, γ-lactone	Rbt	Cl.	O	1 × ENT	Hertz and Tullner (1958)
Estr-4-en-3-one, 4-chloro-17α-ethyl-17β-hydroxy-	Rbt	Cl.	SC	Low activity	Mancera and Ringold (1959)
	Rbt	Cl.	O	Low activity	Mancera and Ringold (1959)
Estr-4-en-3-one, 4-chloro-17α-ethynyl-17β-hydroxy-	Rbt	Cl.	SC	Low activity	Mancera and Ringold (1959)
	Rbt	Cl.	O	Low activity	Mancera and Ringold (1959)
Estr-4-en-3-one, 4-chloro-17β-hydroxy-17α-	Rbt	Cl.	SC	Low activity	Mancera and Ringold (1959)
methyl-	Rbt	Cl.	O	Low activity	Mancera and Ringold (1959)
Estr-4-en-3-one, 4-chloro-17β-hydroxy-17α-vinyl-	Rbt	Cl.	SC	Low activity	Mancera and Ringold (1959)
	Rbt	Cl.	O	Low activity	Mancera and Ringold (1959)
Estr-4-en-3-one, 17α-ethyl-11β,17β-dihydroxy-	Rbt	McG.	IU	< 0.01 × P	Saunders *et al.* (1957a)
Estr-4-en-3-one, 17α-ethyl-17β-hodroxy-	Rbt	Cl.	SC	5–10 × P	Drill and Riegel (1958),

				Edgren *et al.* (1959), Pincus *et al.* (1956b), Saunders *et al.* (1957a), Saunders (1958), Saunders and Drill (1958), Zarrow *et al.* (1958)
Rbt	Cl.	O	2.5 × P (SC)	Drill and Riegel (1958)
Rbt	Cl.	O	2.5 × NP	Saunders and Drill (1958)
Rbt	McG.	IU	0.25 × P	Drill and Riegel (1958), Saunders (1958), Saunders and Drill (1958), Saunders *et al.* (1957a)
Rbt	Cl.	IM	10 × P	Pincus *et al.* (1956a)
Rbt	Carb. An.	SC	2.6 × P	Miyake and Pincus (1958)
Rbt	Carb. An.	O	3.2 × AP	Miyake and Pincus (1958)
Rbt	GMR	SC	2.6 × P	Miyake and Pincus (1958)
Rbt	GMR	O	4.4 × AP	Miyake and Pincus (1958)
R	Decid.	—	0.4 × P	Zarrow *et al.* (1958)
R	Decid.	SC	1 × P	Pincus *et al.* (1956a)
M	Decid.	—	1.8 × P	Zarrow *et al.* (1958)
Chick	Oviduct	—	1.8 × P	Zarrow *et al.* (1958)
R	P-M	SC	Active	Stucki (1958), Suchowsky (1962)
R	P-M	SC	2 × P	Saunders and Elton (1959)
Rbt	P-M	SC	0.7–1 × P	Drill and Riegel (1958), Pincus *et al.* (1956a,b), Saunders and Elton (1959)
Rbt	P-M	SC	Low activity	Saunders *et al.* (1957a), Saunders and Drill (1958)
Rbt	P-M	O	Inactive	Saunders and Drill (1958), Saunders and Elton (1959)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Estr-4-en-3-one, 17α-ethyl-17β-hydroxy-	R	Estrus	SC	4 × P	Pincus *et al.* (1956b)
Estr-4-en-3-one, 17α-ethynyl-10β,17β-dihydroxy-	Rbt	Cl.	O	0.25 × ENT	Ruelas *et al.* (1958)
Estr-4-en-3-one, 17α-ethynyl-17β-hydroxy-	Rbt	Cl.	SC	0.5 × P	Bergstrom *et al.* (1960), Drill and Riegel (1958), Edgren *et al.* (1959), Saunders and Drill (1958)
	Rbt	Cl.	SC	5–10 × P	Pincus *et al.* (1956b), Zarrow *et al.* (1958)
	Rbt	Cl.	O	0.5 × P (SC)	Bergstrom *et al.* (1960)
	Rbt	Cl.	O	1 × P (SC)	Drill and Riegel (1958)
	Rbt	Cl.	O	Several × P (O)	Djerassi *et al.* (1954)
	Rbt	Cl.	O	1 × NP	Saunders and Drill (1958)
	Rbt	Cl.	O	8 × AP	Arcari *et al.* (1963)
	Rbt	Cl.	O	> −5 × ET	Djerassi *et al.* (1954), Hertz *et al.* (1954), McGinty and Djerassi (1958)
	Rbt	Cl.	O	16 × ET	Madjerek *et al.* (1960)
	Rbt	Cl.	O	Active	Andreoli (1962)
	Rbt	Cl.	IM	Same as oral	McGinty and Djerassi (1958)
	Rbt	Cl.	IM	10 × P	Pincus *et al.* (1956a)
	Rbt	McG.	IU	< 0.01 × P	Saunders *et al.* (1957a), Drill and Riegel (1958)
	Rbt	McG.	IU	Inactive	McGinty and Djerassi (1958), Saunders and Drill (1958)
	Rbt	Carb. An.	—	Active	Lutwak-Mann and Adams (1957)
	Rbt	Carb. An.	SC	0.1 × P	Miyake and Pincus (1958)
	Rbt	Carb. An.	O	1.0 × AP	Miyake and Pincus (1958)
	Rbt	GMR	SC	0.2 × P	Miyake and Pincus (1958)

	Rbt	GMR	O	$0.5 \times$ AP	Miyake and Pincus (1958)
	M	Decid.	—	Good	Madjerek *et al.* (1960)
	M	Decid.	—	$< 0.5 \times$ P	Zarrow *et al.* (1958)
	R	Decid.	—	$0.7 \times$ P	Zarrow *et al.* (1958)
	R	Decid.	SC	Inactive	Pincus *et al.* (1956a)
	—	Decid.	O	$\pm$	Andreoli (1962)
	R	P-M	SC	Inactive	Stucki (1958)
	R	P-M	—	Poor	Madjerek *et al.* (1960)
	Rbt	P-M	SC	Inactive	Pincus *et al.* (1956a,b)
	Rbt	P-M	O	Inactive	Andreoli (1962)
	R	P-M	SC	$<$ P	Saunders and Elton (1959)
	Rbt	P-M	O	$< 0.1 \times$ P (SC)	Saunders and Elton (1959)
	Chick	Oviduct	—	$15 \times$ P	Zarrow *et al.* (1958)
	R	Estrus	SC	Inactive	Pincus *et al.* (1956b)
	Mky	EIWB	O	Active	Tullner and Hertz (1957)
Estr-4-en-3-one, 17α-ethynyl-17β-hydroxy-acetate	Rbt	Cl.	O	$3 \times$ ENT	Engelfried *et al.* (1957, 1960)
	Rbt	Cl.	SC	$30 \times$ P (duration $= 1.4 \times$ P)	Engelfried *et al.* (1960)
	R	Inhibit estrus	O	Active	Falconi and Ercoli (1961)
Estr-4-en-3-one, 17α-ethynyl-17β-hydroxy-, butyrate	Rbt	Cl.	SC	$20 \times$ P	Engelfried *et al.* (1960)
Estr-4-en-3-one, 17α-ethynyl-17β-hydroxy-, caproate	Rbt	Cl.	SC	$60 \times$ P (duration $= 1.6 \times$ P)	Engelfried *et al.* (1960)
	Rbt	Cl.	O	$1.2 \times$ ENT	Engelfried *et al.* (1960)
Estr-4-en-3-one, 17α-ethynyl-17β-hydroxy-, caprylate	Rbt	Cl.	SC	Duration $= 2.4 \times$ P	Engelfried *et al.* (1960)
Estr-4-en-3-one, 17α-ethynyl-17β-hydroxy-, enanthate	Rbt	Cl.	SC	Duration $= 3 \times$ P	Engelfried *et al.* (1960)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Estr-4-en-3-one, 17α-ethynyl-17β-hydroxy, undecylate	Rbt	Cl.	SC	Duration = 2.4 × P	Engelfried *et al.* (1960)
Estr-4-en-3-one, 17α-ethynyl-17β-hydroxy-, valerate	Rbt	Cl.	SC	20 × P (duration = 1.6 × P)	Engelfried *et al.* (1960)
	Rbt	Cl.	O	1.2 × ENT	Engelfried *et al.* (1960)
Estr-4-en-3-one, 17α-ethynyl-17β-hydroxy-1-methyl-	Rbt	Cl.	O	1 × ENT	Ringold *et al.* (1956b)
Estr-4-en-3-one, 17β-hydroxy-	Rbt	Cl.	SC	Inactive	Edgren *et al.* (1959)
	Rbt	Cl.	SC	< 0.1 × P	Saunders *et al.* (1957a)
	Rbt	Cl.	SC	< 0.005 × P	Drill and Riegel (1958)
	Rbt	Cl.	O	< 0.2 × P (SC)	Drill and Riegel (1958)
	Rbt	McG.	IU	< 0.005 × P	Drill and Riegel (1958)
	Rbt	McG.	IU	< 0.01 × P	Saunders *et al.* (1957a)
	Rbt	P-M	SC	Inactive	Saunders *et al.* (1957a), Saunders and Drill (1958)
Estr-4-en-3-one, 17β-hydroxy-17α-methyl-	Rbt	Cl.	SC	0.3 × P	Edgren *et al.* (1959)
	Rbt	Cl.	SC	2.5 × P	Saunders *et al.* (1957a)
	Rbt	Cl.	SC	5–10 × P	Drill and Riegel (1958), Pincus *et al.* (1956b)
	Rbt	Cl.	O	1 × ENT	Madjerek *et al.* (1960), McGinty and Djerassi (1958)
	Rbt	Cl.	O	1 × P (SC), 50 × ET	Drill and Riegel (1958), Ferin (1956), Overbeek and de Visser (1956)
	Rbt	Cl.	O	Active	Andreoli (1962)
	Rbt	Cl.	IM	< P	Moggian (1959)

	Rbt	Cl.	IM	1 × ENT	McGinty and Djerassi (1958)
	Rbt	Cl.	IM	10 × P	Pincus *et al.* (1956a)
	Rbt	McG.	IU	< 0.01 × P	Drill and Riegel (1958), Saunders *et al.* (1957a)
	Rbt	Carb. An.	SC	0.1 × P	Miyake and Pincus (1958)
	Rbt	Carb. An.	O	1.5 × AP	Miyake and Pincus (1958)
	Rbt	GMR	SC	0.1 × P	Miyake and Pincus (1958)
	Rbt	GMR	O	2.0 × AP	Miyake and Pincus (1958)
	R	Decid.	SC	1 × P	Pincus *et al.* (1956a)
	—	Decid.	O	Active	Andreoli (1962)
	Rbt	P-M	SC	Inactive	Drill and Riegel (1958), Saunders and Drill (1958)
	Rbt	P-M	—	Slight activity	Overbeek and de Visser (1956), Saunders *et al.* (1957a)
	R	P-M	SC	Active	Suchowsky (1962)
	R	P-M	—	Slight activity	Madjerek *et al.* (1960), Overbeek and de Visser (1956)
	—	P-M	O	±	Andreoli (1962)
	R	Estrus	SC	1 × P	Pincus *et al.* (1956b)
Estr-4-en-3-one, 17β-hydroxy-17α-(2-methallyl)-	Rbt	Cl.	SC	12 × P	Edgren *et al.* (1959)
	Rbt	Cl.	SC	25 × P	Elton and Edgren (1958), Saunders (1958)
	Rbt	McG.	IU	10 × P	Elton and Edgren (1958), Saunders (1958)
	Rbt	Cl.	O	10 × P (SC)	Elton and Edgren (1958)
	Rbt	Carb. An.	SC	10 × P	Elton and Edgren (1958)
	Rbt	Carb. An.	O	2–3 × P (SC)	Elton and Edgren (1958)
	R	Decid.	SC	10 × P	Elton and Edgren (1958)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Estr-4-en-3-one, 17β-hydroxy-17α-(2-methallyl)-	Rbt	P-M	SC	Active	Elton and Edgren (1958)
	R	P-M	SC	10 × P	Saunders and Elton (1959)
	R	P-M	O	> P (SC)	Saunders and Elton (1959)
	Rbt	P-M	SC	> 4 × P	Saunders and Elton (1959)
Estr-4-en-3-one, 17β-hydroxy-17α-(1-methyl-2-propenyl)-	Rbt.	Cl.	SC	5 × P	Drill and Riegel (1958), Edgren *et al.* (1959), Saunders (1958), Saunders and Drill (1958)
	Rbt	Cl.	O	0.5 × P (SC)	Drill and Riegel (1958)
	Rbt	Cl.	O	1 × NP (O)	Saunders and Drill (1958)
	Rbt	McG.	IU	10 × P	Drill and Riegel (1958), Saunders and Drill (1958)
	Rbt	P-M	SC	Active (> P)	Drill and Riegel (1958), Saunders and Drill (1958), Saunders and Elton (1959)
Estr-4-en-3-one, 17β-hydroxy-17α-octyl-	Rbt	Cl.	SC	0.05–0.1 × P	Drill and Riegel (1958)
	Rbt	Cl.	SC	< 0.25 × P	Saunders *et al.* (1957a)
	Rbt	McG.	IU	0.5–1 × P	Drill and Riegel (1958), Saunders *et al.* (1957a)
Estr-4-en-3-one, 17β-hydroxy-17α-(2-propenyl)-	Rbt	Cl.	SC	5 × P	Edgren *et al.* (1959), Saunders *et al.* (1957a)
	Rbt	Cl.	SC	10 × P	Drill and Riegel (1958)
	Rbt	Cl.	O	2.5 × P (SC)	Drill and Riegel (1958)
	Rbt	Cl.	O	2.18 × AP	Arcari *et al.* (1963)
	Rbt	McG.	IU	0.5–1 × P	Drill and Riegel (1958), Saunders *et al.* (1957a)
	Rbt	Carb. An.	SC	5.8 × P	Miyake and Pincus (1958)
	Rbt	Carb. An.	O	1.3 × AP	Miyake and Pincus (1958)

	Rbt	GMR	SC	2.7 × P	Miyake and Pincus (1958)
	Rbt	GMR	O	1.1 × AP	Miyake and Pincus (1958)
	R	P-M	SC	Active	Saunders and Elton (1959)
Estr-4-en-3-one, 17β-hydroxy-17α-isopropyl-	Rbt	Cl.	SC	20 × P	Edgren *et al.* (1959)
Estr-4-en-3-one, 17β-hydroxy-17α-propyl-	Rbt	Cl.	SC	2–3 × P	Drill and Riegel (1958), Edgren *et al.* (1959), Saunders *et al.* (1957a)
	Rbt	Cl.	O	0.25 × P (SC)	Drill and Riegel (1958)
	Rbt	McG.	IU	5 × P	Drill and Riegel (1958), Saunders *et al.* (1957a)
	Rbt	P-M	SC	Active (sl. < P)	Drill and Riegel (1958), Saunders *et al.* (1957a)
	Rbt	Carb. An.	SC	2.9 × P	Miyake and Pincus (1958)
	Rbt	Carb. An.	O	1.7 × AP	Miyake and Pincus (1958)
	Rbt	GMR	SC	2.6 × P	Miyake and Pincus (1958)
	Rbt	GMR	O	1.2 × AP	Miyake and Pincus (1958)
Estr-4-en-3-one, 17β-hydroxy-17α-propynyl-	Rbt	Cl.	SC	5 × P	Edgren *et al.* (1959)
	Rbt	Cl.	O	1.2 × ENT	Knox *et al.* (1960)
Estr-4-en-3-one, 17β-hydroxy-17α-vinyl-	Rbt	Cl.	SC	> 0.5 × P	Saunders *et al.* (1957a)
	Rbt	Cl.	SC	1.5 × P	Edgren *et al.* (1959)
	Rbt	Cl.	SC	5 × P	Drill and Riegel (1958)
	Rbt	Cl.	O	2.5 × P (SC)	Drill and Riegel (1958)
	Rbt	Cl.	O	1 × ENT	McGinty and Djerassi (1958)
	Rbt	Cl.	IM	1 × ENT	McGinty and Djerassi (1958)
	Rbt	McG.	IU	< 0.01 × P	Drill and Riegel (1958), Saunders *et al.* (1957a)
Estr-4-en-3-one, 17β-hydroxymethyl-	Rbt	Cl.	SC	1 × P	Moggian (1958)
Estra-4,6-diene-3β,17β-diol, 17α-ethynyl-, diacetate	Rbt	Cl.	SC	1 × P	Baran (1963)
Estra-4,6-diene-3β,17β-diol, 17α-ethynyl-,	Rbt	Cl.	SC	1 × P	Baran (1963)
17-acetate	Rbt	Cl.	O	0.1 × P (SC)	Baran (1963)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Estra-4,6-dien-3-one, 17α-(1-butynyl)-17β-hydroxy-, acetate	Rbt	Cl.	O	Long acting	Colton (1960)
Estra-4,6-dien-3-one, 6-chloro-17α-ethynyl-17β-hydroxy-	Rbt	Cl.	O	2 × ENT	Knox *et al.* (1960)
	Rbt	Cl.	O	> ENT	Kincl (1961)
Estra-4,6-dien-3-one, 6-chloro-17α-ethynyl-17β-hydroxy-, acetate	Rbt	Cl.	O	< ENT	Kincl (1961)
Estra-4,6-dien-3-one, 17α-ethynyl-17β-hydroxy-	Rbt	Cl.	O	0.4 × ENT	Knox *et al.* (1960)
	Rbt	Cl.	O	Sl. < ENT	Kincl (1961)
Estra-4,6-dien-3-one, 17α-ethynyl-17β-hydroxy-, acetate	Rbt	Cl.	O	Long acting	Colton (1960)
	Rbt	Cl.	SC	2.5 × P	Baran (1963)
	Rbt	Cl.	O	1 × P (SC)	Baran (1963)
	Rbt	Cl.	O	1 × Δ^6-ENT	Kincl (1961)
Estra-4,6-dien-3-one, 17β-hydroxy-6-methyl-17α-(1-propynyl)-, acetate	Rbt	Cl.	O	Long acting	Colton (1960)
Estra-4,6-dien-3-one, 17β-hydroxy-17α-(1-propynyl)-, acetate	Rbt	Cl.	O	Long acting	Colton (1960)
Estra-4,9-dien-3-one, 17α-ethynyl-17β-hydroxy-	R	P-M	—	> ENT	Perelman *et al.* (1960)
Estr-5-en-3-one, 17α-ethyl-17β-hydroxy-	—	—	O	1 × ethyl-NT	Iriarte *et al.* (1959)
Estr-5-en-3-one, 17α-ethynyl-17β-hydroxy-	—	—	O	1 × ENT	Iriarte *et al.* (1959)
Estr-5-en-3-one, 17β-hydroxy-17α-methyl-	—	—	O	1 × methyl-NT	Iriarte *et al.* (1959)
Estr-5(10)-en-3-one, 17α-ethyl-17β-hydroxy-	Rbt	Cl.	SC	0.5 × P	Edgren *et al.* (1959)
	Rbt	Cl.	SC	0.1–0.25 × P	Drill and Riegel (1958)
	Rbt	Cl.	O	0.25 × P (SC)	Drill and Riegel (1958)
	Rbt	McG.	IU	0.25–0.5 × P	Drill and Riegel (1958)
Estr-5(10)-en-3-one, 17α-ethynyl-17β-hydroxy-	Rbt	Cl.	SC	Sl. activity	Drill and Saunders (1957), Edgren *et al.* (1959), Saunders and Drill (1958)
	Rbt	Cl.	SC	< P	Saunders *et al.* (1957b)

Rbt	Cl.	SC	0.0–0.5 × P	Drill and Riegel (1958), Pincus *et al.* (1956b), Saunders (1958)
Rbt	Cl.	O	10–25 × P (O)	Saunders *et al.* (1957b), Saunders and Drill (1958)
Rbt	Cl.	O	0.25 × P (SC)	Drill and Riegel (1958)
Rbt	Cl.	O	High activity > P (SC)	Drill and Saunders (1957)
Rbt	Cl.	O	Active	Andreoli (1962)
Rbt	Cl.	IM	0.5 × P	Pincus *et al.* (1956a)
Rbt	McG.	IU	Inactive	Drill and Riegel (1958), Drill and Saunders (1957), Saunders (1958), Saunders *et al.* (1957b), Saunders and Drill (1958)
Rbt	Carb. An.	SC	Inactive	Miyake and Pincus (1958)
Rbt	Carb. An.	O	0.3 × AP	Miyake and Pincus (1958)
Rbt	GMR	SC	Inactive	Miyake and Pincus (1958)
Rbt	GMR	O	0.5 × AP	Miyake and Pincus (1958)
M	H-F	IU	Inactive	Nakao *et al.* (1958)
Rbt	P-M	SC	Inactive	Drill and Riegel (1958), Pincus *et al.* (1956a,b), Saunders and Elton (1959), Saunders and Drill (1958)
Rbt	P-M	O	Inactive	Drill and Riegel (1958), Saunders and Elton (1959), Saunders and Drill (1958)
—	P-M	O	Inactive	Andreoli (1962)
R	P-M	SC	Inactive	Stucki (1958), Saunders and Elton (1959)
R	P-M	O	Inactive	Saunders and Elton (1959)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Estr-5(10)-en-3-one, 17α-ethynyl-17β-hydroxy-	R	Decid.	SC	Inactive	Pincus *et al.* (1956a)
	—	Decid.	O	±	Andreoli (1962)
	R	Estrus	SC	Inactive	Pincus *et al.* (1956b)
Estr-5(10)-en-3-one, 17β-hydroxy-	Rbt	Cl.	SC	Sl. activity	Edgren *et al.* (1959)
Estr-5(10)-en-3-one, 17β-hydroxy-17α-methyl-	Rbt	Cl.	SC	Sl. activity	Edgren *et al.* (1959)
	Rbt	Cl.	SC	0.05 × P	Drill and Riegel (1958)
	Rbt	Cl.	O	0.25 × P (SC)	Drill and Riegel (1958)
	Rbt	McG.	IU	< 0.005 × P	Drill and Riegel (1958)
	R	P-M	SC	Inactive	Saunders and Elton (1959)
Estr-5(10)-en-3-one, 17β-hydroxy-17α-propyl-	Rbt	Cl.	SC	0.5 × P	Drill and Riegel (1958), Edgren *et al.* (1959)
	Rbt	Cl.	O	0.1 × P (SC)	Drill and Riegel (1958)
	Rbt	McG.	IU	0.25 × P	Drill and Riegel (1958)
Estr-5(10)-en-3-one, 17β-hydroxy-17α-vinyl-	Rbt	Cl.	SC	0.25 × P	Drill and Riegel (1958)
	Rbt	McG.	IU	< 0.005 × P	Drill and Riegel (1958)
D-Homo-17aα-5β-pregna-11,20-dione, 3α,17aβ-dihydroxy-	—	—	—	Inactive	Clinton *et al.* (1958)
D-Homo-5β-pregna-3,11,20-trione, 17aα,21-dihydroxy-, 21-acetate	—	—	—	Inactive	Clinton *et al.* (1958)
D-Homo-5β-pregna-11,20-dione, 3α,17aα,21-trihydroxy-, 3,21-diacetate	—	—	—	Inactive	Clinton *et al.* (1958)
Norchol-4-ene-3,22-dione	Rbt	C-A	—	Inactive	Wettstein (1941)
Norchol-4-ene-3,22-dione, 23-hydroxy-, acetate	Rbt	C-A	—	Inactive	Wettstein (1941)
A-Norpregn-4-ene-3,20-dione	—	—	—	Inactive	Lerner *et al.* (1960)
3,5-Seco-*A*-norpregn-5-en-20-one, 5-hydroxy-3-oic-3,5-lactone	M	H-F	IU	0.0012 × P	Zarrow *et al.* (1957)
3,5-Seco-*A*-norpregna-5,20-dien-3-oic acid	M	H-F	IU	0.0003 × P	Zarrow *et al.* (1957)
18-Nor-13α,17α-pregn-4-ene-3,20-dione	Rbt	Cl.	SC	0.1 × P	Anliker *et al.* (1960)

18-Norpregn-4-ene-3,20-dione	Rbt	Cl.	SC	0.25 × P	Anliker *et al.* (1960)
18,19-Dinorpregn-4-ene-3,20-dione	Rbt	Cl.	SC	0.1 × P	Anliker *et al.* (1960), Johns (1958)
	Rbt	McG.	IU	Sl. activity (< P)	Salhanick and Swanson (1960, 1961)
	—	—	—	Inactive	Stork *et al.* (1958)
dl-18,19-Dinorpregn-4-ene-3,20-dione	—	—	—	Inactive	Stork *et al.* (1958)
19-Nor-5α-pregnane-3,20-dione	Rbt	Cl.	SC	≦ 0.05 × P	Kincl and Folch-Pi (1962b)
19-Norpregna-1,3,5(10)-trien-20-one, 3-hydroxy-	M	H-F	IU	0.0003 × P	Zarrow *et al.* (1957)
	Rbt	Cl.	SC	Inactive	Djerassi *et al.* (1953)
19-Norpregna-1,3,5(10)-trien-20-one, 3,17α-dihydroxy-	M	H-F	IU	0.003 × P	Zarrow *et al.* (1957)
19-Norpregna-3,5-dien-20-one	Rbt	Cl.	SC	< 0.08 × P	Kincl and Folch-Pi (1962b)
19-Norpregn-3,5-dien-20-one, 3-ethoxy-	Rbt	Cl.	O	0.1 × ENT	Kincl and Folch-Pi (1962a)
19-Nor-10ε,14β-pregn-4-ene-3,20-dione	Rbt	Cl.	SC	8 × P	Barber and Ehrenstein (1957)
	Rbt	Cl.	SC	1 × NP	Ehrenstein *et al.* (1957)
19-Nor-10ε,14β,17α-pregn-4-ene-3,20-dione	Rbt	Cl.	SC	1 × NP	Ehrenstein *et al.* (1957)
	Rbt	Cl.	SC	8 × P	Barber and Ehrenstein (1957)
19-Norpregn-4-ene-3,20-dione	Rbt	Cl.	SC	1 × P	Meystre *et al.* (1948), Miramontes *et al.* (1951)
	Rbt	Cl.	SC	4–8 × P	Djerassi *et al.* (1953, 1954), Kincl and Folch-Pi (1962b), Tullner and Hertz (1952, 1953)
	Rbt	Cl.	SC	10 × P	Saunders and Drill (1958)
	Rbt	Cl.	O	20 × P	Saunders and Drill (1958)
	Rbt	Cl.	O	0.05–0.1 × ENT	Kincl and Folch-Pi (1962a)
	Rbt	McG.	IU	Active	Salhanick and Swanson (1960)
	Rbt	McG.	IU	1 × P	Saunders and Drill (1958)
	Rbt	C-A	—	> P	Allen and Ehrenstein (1944)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
19-Norpregn-4-ene-3,20-dione	Rbt	C-A	SC	$1 \times P$	Meystre *et al.* (1948)
	Rbt	C-A	SC	$4–8 \times P$	Djerassi *et al.* (1953, 1954), Tullner and Hertz (1952, 1953)
	M	H-F	IU	$0.3 \times P$	Zarrow *et al.* (1957)
	Mky	EIWB	SC	$8–16 \times P$	Tullner and Hertz (1957)
19-Norpregn-4-ene-3,20-dione, 4-chloro-	Rbt	Cl.	SC	Low activity	Mancera and Ringold (1959)
	Rbt	Cl.	O	Low activity	Mancera and Ringold (1959)
19-Norpregn-4-ene-3,20-dione, 17α-hydroxy-	Rbt	Cl.	SC	$0.01 \times P$	Kessler and Borman (1957)
	Rbt	Cl.	O	$0.05 \times ENT$	Kincl and Folch-Pi (1962a)
19-Norpregn-4-ene-3,20-dione, 17α-hydroxy-, acetate	R	P-M	—	Inactive	Suchowsky (1962)
19-Norpregn-4-ene-3,20-dione, 17α-hydroxy-, caproate	R	P-M	—	Inactive	Suchowsky (1962)
19-Norpregn-4-ene-3,20-dione, 17α-hydroxy-16α-methyl-, acetate	Rbt	Cl.	O	$\geqq 1 \times ENT$	Kincl and Folch-Pi (1962a)
19-Norpregn-4-ene-3,20-dione, 1-methyl-	Rbt	Cl.	SC	$0.5 \times P$	Djerassi *et al.* (1956a)
19-Norpregn-4-ene-3,20-dione, 1α-methyl-	—	—	—	Active	Ringold (1961)
19-Norpregn-4-ene-3,20-dione, 1β-methyl-	—	—	—	Inactive	Ringold (1961)
19-Norpregn-9-en-17-one, 6β-chloro-3β,17α-dihydroxy-5β-methyl-, diacetate	Rbt	Cl.	—	Inactive	Mihiha (1962)
19-Norpregn-9-en-20-one, 6β-chloro-3β-hydroxy-5β-methyl-, acetate	Rbt	Cl.	—	Inactive	Mihina (1962)
19-Norpregn-9-en-20-one, 6β-fluoro-3β-hydroxy-5β-methyl-, acetate	Rbt	Cl.	—	Inactive	Mihina (1962)
21-Norpregn-4-en-20-al, 3-oxo-	Rbt	—	—	Inactive-weak	Miescher *et al.* (1940a,b)
5β-Pregnane-2α,3α-diol	Rbt	Carb. An.	SC	Inactive	Adams and Lutwak-Mann (1955)

5α-Pregnane-3,20-dione	Rbt	Carb. An.	SC	Inactive	Adams and Lutwak-Mann (1955)
5α-Pregnane-3,20-dione, 21-hydroxy-, acetate	Rbt	C-A	SC	Inactive	Tullner *et al.* (1954)
	Chick	Oviduct	SC	2–4 × P	Tullner *et al.* (1954)
	R	Decid.	SC	Inactive	Tullner *et al.* (1954)
	Mky	EIWB	SC	Inactive	Tullner *et al.* (1954)
5β-Pregnane-3,20-dione	Rbt	Carb. An.	SC	Inactive	Adams and Lutwak-Mann (1955)
	M	H-F	IU	0.00006 × P	Zarrow *et al.* (1957)
5β-Pregnane-3,20-dione, 12α-hydroxy-	Rbt	Carb. An.	—	Inactive	Lutwak-Mann and Adams (1957)
5β-Pregnane-12,20-dione, 3α-hydroxy-	Rbt	Carb. An.	—	Inactive	Lutwak-Mann and Adams (1957)
5β-Pregnan-20-one, 3α-hydroxy-	Rbt	Carb. An.	SC	Inactive	Adams and Lutwak-Mann (1955)
5α-Pregn-1-ene-3,20-dione	Rbt	Cl.	—	Inactive	Butenandt and Mamoli (1935), Schutt and Tamm (1958)
Pregna-1,4-diene-3,11,20-trione, 17α,21-dihydroxy-	Rbt	Cl.	SC	Inactive	Hertz and Tullner (1956)
Pregna-1,4-diene-3,20-dione	Rbt	Cl.	O	Sl. < P	Ringold *et al.* (1959b)
Pregna-1,4-diene-3,20-dione, 6α-bromo-17α-hydroxy-, acetate	Rbt	Cl.	O	5–6 × ENT	Ringold *et al.* (1959a), Kincl (1961)
Pregna-1,4-diene-3,20-dione, 6β-bromo-17α-hydroxy-, acetate	Rbt	Cl.	O	0.12–0.25 × 6α-bromo-Δ^1-AP	Kincl (1961)
Pregna-1,4-diene-3,20-dione, 17α-bromo-6α-fluoro-	Rbt	Cl.	SC	10 × P	Chappel *et al.* (1960)
	Rbt	Cl.	O	1 × ENT	Chappel *et al.* (1960)
	Rbt	McG.	IU	Active	Chappel *et al.* (1960)
	R	P-M	—	> P	Chappel *et al.* (1960)
Pregna-1,4-diene-3,20-dione, 6α-chloro-17α-hydroxy-, acetate	—	—	—	Active	Zderic (1963)
	Rbt	Cl.	SC	Approx. = AP	Kincl (1961)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Pregna-1,4-diene-3,20-dione, 6α-chloro-17α-hydroxy-, acetate	Rbt	Cl.	O	6–8 × ENT	Ringold *et al.* (1959a), Kincl (1961)
Pregna-1,4-diene-3,20-dione, 9α-chloro-11β-fluoro-	Rbt	Cl.	SC	2 × P	Reiman *et al.* (1960)
Pregna-1,4-diene-3,20-dione, 9α,11β-dichloro-	Rbt	Cl.	SC	3.0 × P	Reiman *et al.* (1960)
	Rbt	Cl.	SC	0.5 × 9α,11β-dichloro-P	Reiman *et al.* (1961)
Pregna-1,4-diene-3,20-dione-9α,11β-dichloro-17α-hydroxy-, acetate	Rbt	Cl.	SC	0.5 × 9α,11β-dichloro-P acetate	Reiman *et al.* (1961)
Pregna-1,4-diene-3,20-dione, 6α-fluoro-	Rbt	Cl.	SC	< 10 × P	Deghenghi *et al.* (1963b)
	Rbt	Cl.	O	< 0.3 × ENT	Deghenghi *et al.* (1963b)
Pregna-1,4-diene-3,20-dione, 6α-fluoro-17α-hydroxy-, acetate	Rbt	Cl.	O	6 × ENT	Bowers *et al.* (1959b), Ringold *et al.* (1959a)
Pregna-1,4-diene-3,20-dione, 6α-fluoro-17α-methyl-	Rbt	Cl.	SC	< 5 × P	Deghenghi *et al.* (1963a)
	Rbt	Cl.	O	0.3 × ENT	Deghenghi *et al.* (1963a)
Pregna-1,4-diene-3,20-dione, 9α-fluoro-11β,17α,21-trihydroxy-, 21-acetate	Rbt	Cl.	SC	< 0.01 × P	Hertz and Tullner (1956)
Pregna-1,4-diene-3,20-dione, 21-fluoro-17α-hydroxy-, acetate	Rbt	Cl.	SC	5 × P	Bergstrom *et al.* (1960)
Pregna-1,4-diene-3,20-dione, 21-fluoro-17α-methyl-	Rbt	Cl.	SC	< 5 × P	Deghenghi *et al.* (1963a)
	Rbt	Cl.	O	< 1 × ENT	Deghenghi *et al.* (1963a)
Pregna-1,4-diene-3,20-dione, 11β,17α,21-trihydroxy-	Rbt	Cl.	SC	Inactive	Hertz and Tullner (1956)
Pregna-1,4-diene-3,20-dione, 17α-hydroxy-, acetate	Rbt	Cl.	—	< P	Wada (1959)
	Rbt	Cl.	SC	Potent	Babcock and Pederson (1961)
	Rbt	Cl.	O	Active	Babcock and Pederson (1961)
	Rbt	Cl.	O	0.5 × ENT	Kincl (1961)
	Rbt	C-A	—	Sl. > AP	Wada (1959)

Pregna-1,4-diene-3,20-dione, 17α-hydroxy-, caproate	Rbt	Cl.	—	<P	Wada (1959)
	Rbt	C-A	—	Sl. > 17α-OH-P caproate	Wada (1959)
Pregna-1,4-diene-3,20-dione, 17α-hydroxy-6α-methyl-, acetate	Rbt	Cl.	O	8 × ENT	Ringold *et al.* (1959b), Kincl (1961)
Pregna-1,4-diene-3,20-dione, 17α-hydroxy-16α-methyl-, acetate	Rbt	Cl.	O	0.3 × ENT	Kincl and Folch-Pi (1962a)
	Rbt	Cl.	O	8 × AP	Bernstein *et al.* (1961b)
Pregna-1,4-diene-3,20-dione, 17α,21-dihydroxy-, diacetate	Rbt	Cl.	O	0.4 × DAP	Kincl (1962)
Pregna-1,4,6-triene-3,20-dione, 6-chloro-17α-hydroxy-, acetate	—	—	—	Active	Zderic (1963)
	Rbt	Cl.	O	30–35 × ENT	Ringold *et al.* (1959a), Kincl (1961)
Pregna-1,4,6-triene-3,20-dione, 6-chloro-17α,21-dihydroxy-, diacetate	Rbt	Cl.	O	1 × ENT	Ringold *et al.* (1959a)
	Rbt	Cl.	O	32 × DAP	Kincl (1962)
Pregna-1,4,6-triene-3,20-dione, 6-fluoro-17α-hydroxy-, acetate	Rbt	Cl.	O	6–8 × ENT	Bowers *et al.* (1959b), Kincl (1961)
Pregna 1,4,6-triene-3,20-dione, 17α-hydroxy-, acetate	Rbt	Cl.	O	20 × AP	Dusza *et al.* (1963)
	Rbt	Cl.	O	23 × AP	Hiersemann *et al.* (1960)
Pregna-1,4,6-triene-3,20-dione, 17α-hydroxy-6-methyl-, acetate	Rbt	Cl.	O	10 × ENT	Kincl (1961), Ringold *et al.* (1959b)
Pregna-1,4,6-triene-3,20-dione, 17α-hydroxy-16α-methyl-, acetate	Rbt	Cl.	O	66 × AP	Bernstein *et al.* (1961b)
Pregna-1,4,6,11-tetraene-3,20-dione, 17α-hydroxy-, acetate	Rbt	Cl.	O	10 × AP	Dusza *et al.* (1963)
Pregna-1,4,11-triene-3,20-dione-, 17α-hydroxy-, acetate	Rbt	Cl.	O	8 × AP	Dusza *et al.* (1963)
5α-Pregn-2-ene-1,20-dione	Rbt	Cl.	—	Inactive	Schutt and Tamm (1958)
Pregna-2,5-dien-20-one, 17α-hydroxy-, acetate	Rbt	Cl.	SC	0.04 × P	Kincl and Folch-Pi (1962b)
Pregna-3,5-dien-20-one, 3-cyclopentoxy-	Rbt	Cl.	O	10 × P	Ercoli and Gardi (1960)
	Rbt	Cl.	O	Active	Andreoli (1962)
	—	P-M	O	Active	Andreoli (1962)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Pregna-3,5-dien-20-one, 3-cyclopentoxy-17α-hydroxy, acetate	Rbt	Cl.	O	1 × P (SC)	Falconi *et al.* (1961)
	Rbt	Cl.	O	2.07 × AP	Arcari *et al.* (1963)
	Rbt	Cl.	O	Active	Andreoli (1962)
	—	P-M	O	Active	Andreoli (1962)
	Rbt	P-M	O	> P (SC)	Falconi *et al.* (1961)
	Rbt	McG.	IU	< P or AP	Falconi *et al.* (1961)
	R	Estrus	O	Inactive	Falconi and Ercoli (1961)
Pregna-3,5-dien-20-one, 3-cyclopentoxy-17α-hydroxy-6-methyl-, acetate	R	Estrus	O	Active	Falconi and Ercoli (1961)
	Rbt	Cl.	—	1 × 6α-methyl-AP	Falconi and Ercoli (1961)
	R	P-M	—	1 × 6α-methyl-AP	Falconi and Ercoli (1961)
Pregna-3,5-dien-20-one, 3-hydroxy-, acetate	Rbt	Carb. An.	—	Active	Lutwak-Mann and Adams (1957)
	Rbt	P-M	—	Active	Lutwak-Mann and Adams (1957)
Pregna-3,5-dien-20-one, 3-hydroxy-, benzoate	Rbt	Cl.	SC	> P	Junkmann (1954)
Pregna-3,5-dien-20-one, 3-hydroxy, benzyl ether	Rbt	Cl.	SC	1 × P	Junkmann (1954)
Pregna-3,5-dien-20-one, 3-hydroxy-, *n*-butyrate	Rbt	Cl.	SC	1 × P	Junkmann (1954)
Pregna-3,5-dien-20-one, 3-hydroxy-, *n*-caproate	Rbt	Cl.	SC	> P	Junkmann (1954)
Pregna-3,5-dien-20-one, 3-hydroxy-, chloroacetate	Rbt	Cl.	SC	1 × P	Junkmann (1954)
Pregna-3,5-dien-20-one, 3-hydroxy-, *p*-chlorobenzoate	Rbt	Cl.	SC	1 × P	Junkmann (1954)
Pregna-3,5-dien-20-one, 3-hydroxy-, diethylacetate	Rbt	Cl.	SC	1 × P	Junkmann (1954)
Pregna-3,5-dien-20-one, 3-hydroxy-, hexahydrobenzoate	Rbt	Cl.	SC	> P	Junkmann (1954)
Pregna-3,5-dien-20-one, 3-hydroxy-, trimethylacetate	Rbt	Cl.	SC	> P	Junkmann (1954)

Pregna-3,5-dien-20-one, 3-hydroxy-, propionate	Rbt	Cl.	SC	$1 \times P$	Junkmann (1954)
Pregna-3,5-dien-20-one, 3-hydroxy-, *i*-valerate	Rbt	Cl.	SC	$> P$	Junkmann (1954)
Pregna-3,5-dien-20-one, 3-hydroxy-, *n*-valerate	Rbt	Cl.	SC	$> P$	Junkmann (1954)
Pregna-3,5-dien-20-one, 3,17α-dihydroxy-, diacetate	Rbt	Cl.	SC	$< P$	Junkmann (1954)
Pregna-3,5-dien-20-one, 17α-hydroxy-, acetate	Rbt	Cl.	O	$0.6–0.7 \times ENT$	Kincl and Folch-Pi (1962a)
Pregna-3,5,9(11)-trien-20-one, 21-fluoro-3,17α-dihydroxy-, dipropionate	—	—	SC	$10–20 \times P$	Pederson and Babcock (1962)
	—	—	O	Active	Pederson and Babcock (1962)
Pregn-4-en-3-one, 20-acetyl-20-hydroxy-	Rbt	Cl.	SC	Inactive	Sondheimer *et al.* (1959)
Pregn-4-en-3-one, 20-ethyleneketal	Rbt	Cl.	SC	$< P$	Junkmann (1954)
Pregn-4-en-3-one, 20α-hydroxy-	Rbt	Cl.	SC	$0.3–0.5 \times P$	Zander *et al.* (1958)
	M	H-F	IU	$0.2 \times P$	Zander *et al.* (1958)
Pregn-4-en-3-one, 20β-hydroxy-	Rbt	Cl.	SC	$0.1–0.2 \times P$	Zander *et al.* (1958)
	M	H-F	IU	$1 \times P$	Zander *et al.* (1958)
Pregn-4-en-3-one, 20-oxime	Rbt	Cl.	SC	$< P$	Junkmann (1954)
Pregn-4-en-3-one, 20-oximacetate	Rbt	Cl.	SC	$< P$	Junkmann (1954)
Pregn-4-en-3-one, 20-oxime-*n*-butyrate	Rbt	Cl.	SC	$< P$	Junkmann (1954)
Pregn-4-en-3-one, 20a-sulfoxide	Rbt	McG.	IU	$< 0.005 \times P$	Drill and Riegel (1958)
Pregn-4-en-3-one, 20b-sulfoxide	Rbt	McG.	IU	$< 0.005 \times P$	Drill and Riegel (1958)
Pregn-4-en-3-one, 20-thioxo-	Rbt	Cl.	SC	$0.25 \times P$	Drill and Riegel (1958)
	Rbt	McG.	IU	$0.5 \times P$	Drill and Riegel (1958)
Pregn-4-ene-3,6,20-trione	M	H-F	IU	Inactive	Nakao *et al.* (1958)
Pregn-4-ene-3,11,20-trione	Rbt	Cl.	—	Inactive	Byrnes and Shipley (1955)
	—	—	—	$0.03–0.1 \times P$	Mardones *et al.* (1954a)
	Rbt	Cl.	—	$0.17 \times P$	Reichstein and Fuchs (1940)
	M	H-F	IU	$0.006 \times P$	Zarrow *et al.* (1957)
Pregn-4-ene-3,11,20-trione, 9α-bromo-	Rbt	Cl.	SC	$0.5 \times P$	Fried *et al.* (1958)
	Rbt	Cl.	O	$3–5 \times ET$	Fried *et al.* (1958)
	R	P-M	SC	Active	Stucki (1958)
Pregn-4-ene-3,11,20-trione, 12α-bromo-	Rbt	Cl.	SC	$< 0.125 \times P$	Fried *et al.* (1958)
	Rbt	Cl.	O	$< 0.5 \times ET$	Fried *et al.* (1958)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Pregn-4-ene-3,11,20-trione, 9α-chloro-	Rbt	Cl.	SC	0.25 × P	Fried *et al.* (1958)
	Rbt	Cl.	O	1 × ET	Fried *et al.* (1958)
Pregn-4-ene-3,11,20-trione, 12α-chloro-	Rbt	Cl.	SC	0.125 × P	Fried *et al.* (1958)
	Rbt	Cl.	O	0.2 × ET	Fried *et al.* (1958)
Pregn-4-ene-3,11,20-trione, 9α-fluoro-	Rbt	Cl.	SC	0.25 × P	Fried *et al.* (1958)
	Rbt	Cl.	O	2–3 × ET	Fried *et al.* (1958)
Pregn-4-ene-3,11,20-trione, 9α-fluoro-17α-hydroxy-, acetate	Rbt	Cl.	O	10 × P (SC)	Bergstrom *et al.* (1959)
Pregn-4-ene-3,11,20-trione, 12α-fluoro-	Rbt	Cl.	SC	0.5 × P	Fried *et al.* (1958)
Pregn-4-ene-3,11,20-trione, 17α,21-dihydroxy-, 21-acetate	Rbt	Cl.	SC	Inactive	Hertz and Tullner (1956)
Pregn-4-ene-3,11,20-trione, 21-hydroxy-6α-methyl-, formate	—	—	O	10–20 × P	Lincoln and Spero (1961)
Pregn-4-ene-3,12,20-trione	Rbt	Carb. An.	—	Inactive	Lutwak-Mann and Adams (1957)
Pregn-4-ene-3,19,20-trione	Rbt	Cl.	—	0.1 × P	Barber and Ehrenstein (1955)
	Rbt	C-A	—	Inactive	Barber and Ehrenstein (1955)
8α-Pregn-4-ene-3,20-dione	—	—	—	0.3–0.5 × P	Djerassi *et al.* (1957)
8α,9α-Pregn-4-ene-3,20-dione	Rbt	Cl.	SC	0.25–0.5 × P	Djerassi *et al.* (1956b)
	Rbt	McG	IU	1 × P	Djerassi *et al.* (1956b)
9β,10α-Pregn-4-ene-3,20-dione	Rbt	Cl.	SC	5–6 × P	Reerink *et al.* (1960), Schöler (1960)
	Rbt	Cl.	O	Active	Reerink *et al.* (1960)
	Rbt	Cl.	O	Weak activity	Schöler (1960)
	R	P-M	SC	Active	Schöler (1962)
	R	P-M	O	Inactive	Schöler (1962)
9β,10α-Pregn-4-ene-3,20-dione, 17α-hydroxy-, acetate	Rbt	Cl.	SC	28 × P	Schöler (1960)
	Rbt	Cl.	O	Active	Schöler (1960)

14β,17α-Pregn-4-ene-3,20-dione	—	—	—	Inactive	Plattner *et al.* (1948)
17α-Pregn-4-ene-3,20-dione, 14β-hydroxy-	Rbt	C-A	—	Inactive	Lardon (1949)
17α-Pregn-4-ene-3,20-dione, 17β-hydroxy-, acetate	Rbt	McG.	IU	Sl. activity (<P)	Salhanick and Swanson (1961)
	Rbt	Carb. An.	—	Inactive	Lutwak-Mann and Adams (1957)
	M	H-F	IU	$0.3 \times P$	Zarrow *et al.* (1957)
17α-Pregn-4-ene-3,20-dione, 19:8-lacto-	Rbt	Cl.	—	$<0.1 \times P$	Barber and Ehrenstein (1961)
17α-Pregn-4-ene-3,20-dione, 17β-methyl-	Rbt	C-A	—	Inactive	Heusser *et al.* (1950a,b)
17α-Pregn-4-en-20-yn-3-one, 21-ethyl-17β-hydroxy-6α-methyl-	Rbt	Cl.	O	$9 \times ET$	Barton *et al.* (1960)
17α-Pregn-4-en-20-yn-3-one, 17β-hydroxy-6α,21-dimethyl-	Rbt	Cl.	O	$12 \times ET$	Barton *et al.* (1960)
Pregn-4-ene-3,20-dione	Rbt	Cl.	O	$0.01 \times P$ (SC)	Bergstrom *et al.* (1960)
	Rbt	Cl.	O	$0.0025–0.005 \times$ ENT	Kincl and Folch-Pi (1962a)
	Rbt	Carb. An.	SC	Active	Adams and Lutwak-Mann (1955)
	Rbt	Carb. An.	O	Inactive	Miyake and Pincus (1958)
	Rbt	GMR	O	Inactive	Miyake and Pincus (1958)
	M	H-F	IU	Active	Hooker and Forbes (1947)
Pregn-4-ene-3,20-dione, 6-acetyl-	Rbt	Cl.	SC	Inactive	Zderic and Limon (1960)
Pregn-4-ene-3,20-dione, 6β-acetylthio-	—	—	—	Sl. <P	Komeno (1962)
Pregn-4-ene-3,20-dione, 7α-acetylthio-17α-hydroxy-, propionate	Rbt	Cl.	—	$1 \times P$	Dodson and Tweit (1959)
Pregn-4-ene-3,20-dione, 16α,17α-benzylidenedioxy-	Rbt	Cl.	SC	$1–2 \times P$	Lerner *et al.* (1961a,b)
	Rbt	Cl.	O	$1–2 \times ENT$	Lerner *et al.* (1961a)
	—	P-M	—	>P	Lerner *et al.* (1961b)
Pregn-4-ene-3,20-dione, 16α,17α-benzylidenedioxy-6α-fluoro-	Rbt	Cl.	SC	$2–4 \times P$	Lerner *et al.* (1961a)
	Rbt	Cl.	O	$<1–2 \times ENT$	Lerner *et al.* (1961a)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Pregn-4-ene-3,20-dione, 16α,17α-benzylidenedioxy-6β-fluoro-	Rbt	Cl.	SC	Inactive	Lerner *et al.* (1961a)
	Rbt	Cl.	O	Inactive	Lerner *et al.* (1961a)
Pregn-4-ene-3,20-dione, 16α,17α-benzylidenedioxy-6α-methyl-	Rbt	Cl.	SC	< 1–2 × P	Lerner *et al.* (1961a)
	Rbt	Cl.	O	< 1–2 × ENT	Lerner *et al.* (1961a)
Pregn-4-ene-3,20-dione, 16α,17α-benzylidenedioxy-6β-methyl-	Rbt	Cl.	SC	1–2 × P	Lerner *et al.* (1961a)
	Rbt	Cl.	O	1–2 × ENT	Lerner *et al.* (1961a)
Pregn-4-ene-3,20-dione, 4-bromo-	Rbt	—	—	Sl. activity	Ringold *et al.* (1956a)
Pregn-4-ene-3,20-dione, 6α-bromo-17α-hydroxy-, acetate	Rbt	Cl.	O	1 × ENT	Ringold *et al.* (1959a)
	Rbt	Cl.	O	0.7 × ENT	Kincl (1961)
Pregn-4-ene-3,20-dione, 6β-bromo-17α-hydroxy-, acetate	Rbt	Cl.	O	0.5 × 6α-bromo-AP	Kincl (1961)
Pregn-4-ene-3,20-dione, 9α-bromo-4-chloro-11β-hydroxy-	Rbt	Cl.	SC	0.1 × P	Kincl and Dorfman (1961)
Pregn-4-ene-3,20-dione, 9α-bromo-11β-chloro-	Rbt	Cl.	SC	1 × P	Reiman *et al.* (1960)
Pregn-4-ene-3,20-dione, 9α-bromo-11β-fluoro-	Rbt	Cl.	SC	0.7 × P	Reiman *et al.* (1960)
	Rbt	Cl.	O	< I × P(SC)	Bergstrom and Nicholson (1960)
Pregn-4-ene-3,20-dione, 9α-bromo-11β-fluoro-17α-hydroxy-, acetate	Rbt	Cl.	O	5 × P (SC)	Bergstrom and Nicholson (1960)
Pregn-4-ene-3,20-dione, 9α-bromo-11β-hydroxy-	Rbt	Cl.	SC	0.5 × P	Fried *et al.* (1958)
	Rbt	Cl.	O	2–3 × ET	Fried *et al.* (1958)
Pregn-4-ene-3,20-dione, 9α-bromo-11β,17α-dihydroxy-, 17-acetate	Rbt	Cl.	O	5 × P (SC)	Bergstrom *et al.* (1959)
Pregn-4-ene-3,20-dione, 12α-bromo-11β-hydroxy-	Rbt	Cl.	SC	2.0 × P	Fried *et al.* (1958)
	Rbt	Cl.	O	2–3 × ET	Fried *et al.* (1958)
Pregn-4-ene-3,20-dione, 16-bromo-	Rbt	Cl.	SC	< P	Junkmann (1954)
Pregn-4-ene-3,20-dione, 16α-bromo-	Rbt	Cl.	O	< 0.25 × P (SC)	Drill and Riegel (1958)
	Rbt	McG.	IU	1 × P	Drill and Riegel (1958)

Pregn-4-ene-3,20-dione, 17α-bromo-	Rbt	Cl.	SC	2 × P	Engel and Jahnke (1957), Chappel *et al.* (1960), Deghenghi *et al.* (1963b)
	Rbt	Cl.	O	0.02–0.05 × ENT	Chappel *et al.* (1960), Deghenghi *et al.* (1963b)
	Rbt	McG.	IU	Active	Chappel *et al.* (1960)
	R	P-M	—	> P	Chappel *et al.* (1960)
Pregn-4-ene-3,20-dione, 17α-bromo-6α-chloro-	Rbt	Cl.	SC	1 × P	Chappel *et al.* (1960)
	Rbt	Cl.	O	0.2–0.05 × ENT	Chappel *et al.* (1960)
	Rbt	McG.	IU	Active	Chappel *et al.* (1960)
Pregn-4-ene-3,20-dione, 17α-bromo-6α-fluoro-	—	—	—	Marked	Engel and Deghenghi (1960)
	Rbt	Cl.	SC	4 × P	Chappel *et al.* (1960), Deghenghi *et al.* (1963b)
	Rbt	Cl.	O	0.3–0.5 × ENT	Chappel *et al.* (1960), Deghenghi *et al.* (1963b)
	Rbt	McG.	IU	Active	Chappel *et al.* (1960)
	R	P-M	—	> P	Chappel *et al.* (1960)
Pregn-4-ene-3,20-dione, 21-bromo-	Rbt	Cl.	SC	Very weak	Engel and Jahnke (1957)
Pregn-4-ene-3,20-dione, 21-bromo-17α-hydroxy-, acetate	Rbt	Cl.	SC	1 × P	Elton (1959), Bergstrom *et al.* (1960)
	Rbt	Cl.	O	Inactive	Elton (1959), Bergstrom *et al.* (1960)
	Rbt	McG.	IU	1–5 × P	Elton (1959)
	Rbt	Carb. An.	SC	1 × P	Elton (1959)
	Rbt	Carb. An.	O	Very sl. activity	Elton (1959)
Pregn-4-ene-3,20-dione, 4-chloro-	Rbt	—	—	Sl. activity	Ringold *et al.* (1956a)
Pregn-4-ene-3,20-dione, 6α-chloro-17α-bromo-	Rbt	Cl.	O	0.2 × ENT	Mills *et al.* (1960)
Pregn-4-ene-3,20-dione, 6α-chloro-17α-hydroxy-, acetate	Rbt	Cl.	O	2–3 × ENT	Ringold *et al.* (1959a), Kincl (1961)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Pregn-4-ene-3,20-dione, 6α-chloro-17α-hydroxy-, acetate	Rbt	McG.	IU	1 × P	Salhanick and Swanson (1961)
	Rbt	Cl.	SC	> AP	Kincl (1961)
Pregn-4-ene-3,20-dione, 6β-chloro-17α-hydroxy-, acetate	Rbt	Cl.	O	0.2–0.25 × 6α-chloro-AP	Kincl (1961)
Pregn-4-ene-3,20-dione, 6α-chloro-17α,21-dihydroxy-, diacetate	Rbt	Cl.	O	0.5 × ENT	Ringold *et al.* (1959a)
	Rbt	Cl.	O	32 × DAP	Kincl (1962)
Pregn-4-ene-3,20-dione, 6α-chloro-17α-methyl-	Rbt	Cl.	SC	< 5 × P	Deghenghi *et al.* (1963a,b)
	Rbt	Cl.	O	< 1 × ENT	Deghenghi (1963b)
Pregn-4-ene-3,20-dione, 6α,17α-dichloro-	Rbt	Cl.	O	0.2 × ENT	Mills *et al.* (1960)
Pregn-4-ene-3,20-dione, 9α-chloro-11β-fluoro-	Rbt	Cl.	O	1 × P (SC)	Bergstrom and Nicholson (1960)
Pregn-4-ene-3,20-dione, 9α-chloro-11β-hydroxy-	Rbt	Cl.	SC	0.25 × P	Fried *et al.* (1958)
	Rbt	Cl.	O	1 × ET	Fried *et al.* (1958)
Pregn-4-ene-3,20-dione, 9α-chloro-11β,17α,21-trihydroxy-, 21-acetate	Rbt	Cl.	SC	0.03 × P	Hertz and Tulner (1956)
Pregn-4-ene-3,20-dione, 9α,11β-dichloro-	Rbt	Cl.	SC	5.5 × P	Reiman *et al.* (1960)
Pregn-4-ene-3,20-dione, 12α-chloro-11β-hydroxy-	Rbt	Cl.	SC	2.0 × P	Fried *et al.* (1958)
	Rbt	Cl.	O	1 × ET	Fried *et al.* (1958)
Pregn-4-ene-3,20-dione, 16-chloro-	Rbt	Cl.	SC	< P	Junkmann (1954)
Pregn-4-ene-3,20-dione, 16α-chloro-	Rbt	Cl.	SC	0.1 × P	Drill and Riegel (1958)
	Rbt	Cl.	O	< 0.1 × P (SC)	Drill and Riegel (1958)
	Rbt	McG.	IU	0.10–0.25 × P	Drill and Riegel (1958)
Pregn-4-ene-3,20-dione, 16-chloro-17α-hydroxy-	Rbt	Cl.	SC	< P	Junkmann (1954)
Pregn-4-ene-3,20-dione, 16-chloro-17α-hydroxy-, acetate	Rbt	Cl.	SC	< P	Junkmann (1954)
Pregn-4-ene-3,20-dione, 17α-chloro-	Rbt	Cl.	SC	1 × P	Chappel *et al.* (1960)
	Rbt	Cl.	O	0.2–0.05 × ENT	Chappel *et al.* (1960)

Pregn-4-ene-3,20-dione, 21-chloro-	Rbt	Cl.	SC	Inactive	Elton (1959)
	Rbt	Cl.	SC	$< 0.05 \times P$	Drill and Riegel (1958), Engel and Noble (1957)
	Rbt	McG.	IU	$0.05 \times P$	Drill and Riegel (1958), Elton (1959)
Pregn-4-ene-3,20-dione, 21-chloro-17α-hydroxy-	Rbt	Cl.	SC	Inactive	Elton (1959)
	Rbt	Carb. An.	SC	Inactive	Elton (1959)
	Rbt	McG.	IU	Inactive	Elton (1959)
Pregn-4-ene-3,20-dione, 21-chloro-17α-hydroxy-, acetate	Rbt	Cl.	SC	$2 \times P$	Bergstrom *et al.* (1960), Elton (1959)
	Rbt	Cl.	O	$< 0.2 \times P$ (SC)	Bergstrom *et al.* (1960), Elton (1959)
	Rbt	Carb. An.	SC	$2 \times P$	Elton (1959)
	Rbt	Carb. An.	O	$< 0.2 \times P$	Elton (1959)
	Rbt	McG.	IU	$10 \times P$	Elton (1959)
Pregn-4-ene-3,20-dione, 21-chloro-17α-hydroxy-6α-methyl-, acetate	Rbt	Cl.	SC	$2 \times P$	Bergstrom *et al.* (1960), Elton (1959)
	Rbt	Carb. An.	SC	$2 \times P$	Elton (1959)
	Rbt	McG.	IU	$10 \times P$	Elton (1959)
Pregn-4-ene-3,20-dione, 6α-(1-chlorovinyl)-	Rbt	Cl.	SC	Inactive	Zderic and Limon (1960)
Pregn-4-ene-3,20-dione, 8,19-epoxy-	Rbt	Cl.	—	$< 0.1 \times P$	Otto and Ehrenstein (1961)
Pregn-4-ene-3,20-dione, 16α,17α-epoxy-	Rbt	Cl.	SC	$< P$	Junkmann (1954)
Pregn-4-ene-3,20-dione, 17α-ethyl-	Rbt	Cl.	SC	$> 2 \times P$	Deghenghi *et al.* (1963b)
	Rbt	Cl.	O	$0.1 \times ENT$	Deghenghi *et al.* (1963b)
Pregn-4-ene-3,20-dione, 17α-ethyl-6α-methyl-	Rbt	Cl.	SC	$10 \times P$	Deghenghi *et al.* (1963b)
	Rbt	Cl.	O	$5 \times ENT$	Deghenghi *et al.* (1963b)
Pregn-4-ene-3,20-dione, 21-ethyl-	Rbt	Cl.	SC	Inactive	Wettstein (1940b)
	Rbt	Cl.	O	Inactive	Wettstein (1940b)
Pregn-4-ene-3,20-dione, 6α-fluoro-	Rbt	Cl.	SC	$5 \times P$	Deghenghi *et al.* (1963b)
	Rbt	Cl.	O	$< 0.3 \times ENT$	Deghenghi *et al.* (1963b)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Pregn-4-ene-3,20-dione, 6α-fluoro-	Rbt	McG.	IU	1 × P	Salhanick and Swanson (1961)
Pregn-4-ene-3,20-dione, 6β-fluoro-	Rbt	McG.	IU	Sl. active	Salhanick and Swanson (1961)
Pregn-4-ene-3,20-dione, 6α-fluoro-17α-hydroxy-, acetate	Rbt	Cl.	O	1 × ENT	Bowers *et al.* (1959b)
	Rbt	Cl.	O	> 5–10 × P	Hogg *et al.* (1958)
	Rbt	Cl.	O	0.25–1.0 × ENT	Kincl (1961)
Pregn-4-ene-3,20-dione, 6β-fluoro-17α-hydroxy-, acetate	Rbt	Cl.	O	0.1 × 6α-fluoro-AP	Kincl (1961)
Pregn-4-ene-3,20-dione, 6α-fluoro-17α-hydroxy-16α-methyl-, acetate	Rbt	Cl.	O	2 × ENT	Kincl and Folch-Pi (1962a)
Pregn-4-ene-3,20-dione, 6α-fluoro-16α,17α-isopropylidenedioxy-	Rbt	Cl.	SC	1–2 × P	Lerner *et al.* (1961a)
	Rbt	Cl.	O	< 0.125 × ENT	Lerner *et al.* (1961a)
Pregn-4-ene-3,20-dione, 6β-fluoro-16α,17α-isopropylidenedioxy-	Rbt	Cl.	SC	Inactive	Lerner *et al.* (1961a)
	Rbt	Cl.	O	Inactive	Lerner *et al.* (1961a)
Pregn-4-ene-3,20-dione, 6α-fluoro-16α-methyl-	Rbt	Cl.	SC	1 × P	Bernstein *et al.* (1961b)
Pregn-4-ene-3,20-dione, 6α-fluoro-17α-methyl-	Rbt	Cl.	SC	> 5 × P	Deghenghi *et al.* (1963a)
	Rbt	Cl.	O	< 0.3 × ENT	Deghenghi *et al.* (1963a)
Pregn-4-ene-3,20-dione, 6α,21-difluoro-17α-methyl-	Rbt	Cl.	SC	10 × P	Deghenghi *et al.* (1963a)
	Rbt	Cl.	O	< 10 × ENT	Deghenghi *et al.* (1963a)
Pregn-4-ene-3,20-dione, 9α-fluoro-11β-hydroxy-	Rbt	Cl.	SC	0.05–0.1 × P	Hertz and Tullner (1956)
	Rbt	Cl.	SC	0.125 × P	Fried *et al.* (1958)
	Rbt	Cl.	O	3–5 × ET	Fried *et al.* (1958)
Pregn-4-ene-3,20-dione, 9α-fluoro-11β,17α-dihydroxy-, diacetate	Rbt	Cl.	O	10 × P (SC)	Bergstrom *et al.* (1959)
Pregn-4-ene-3,20-dione, 9α-fluoro-11β,17α-dihydroxy-, 17-acetate	Rbt	Cl.	O	25 × P (SC)	Bergstrom *et al.* (1959)

Pregn-4-ene-3,20-dione, 9α-fluoro-11β,17α,21-trihydroxy-, 21-acetate	Rbt	Cl.	SC	0.03 × P	Hertz and Tullner (1956)
	Mky	EIWB	—	Active	Hertz and Tullner (1956)
Pregn-4-ene-3,20-dione, 12α-fluoro-11β-hydroxy-	Rbt	Cl.	SC	1.0 × P	Fried *et al.* (1958)
	Rbt	Cl.	O	0.2 × ET	Fried *et al.* (1958)
Pregn-4-ene-3,20-dione, 21-fluoro-	Rbt	Cl.	SC	1–3 × P	Bergstrom *et al.* (1960), Deghenghi *et al.* (1963b), Drill and Riegel (1958), Elton (1959), Engel and Noble (1957)
	Rbt	Cl.	O	Very sl. active	Elton (1959)
	Rbt	Cl.	O	Active	Engel and Noble (1957)
	Rbt	Cl.	O	0.05 × P (SC)	Drill and Riegel (1958), Bergstrom *et al.* (1960)
	Rbt	Cl.	O	< 0.05 × ENT	Deghenghi *et al.* (1963b)
	Rbt	McG.	IU	5 × P	Drill and Riegel (1958), Elton (1959)
	Rbt	C-A	SC	2–4 × P	Tannhauser *et al.* (1956)
	Rbt	C-A	O	2–4 × P	Tannhauser *et al.* (1956)
Pregn-4-ene-3,20-dione, 21-fluoro-17α-hydroxy-	Rbt	Cl.	SC	Inactive	Elton (1959)
	Rbt	Cl.	O	Inactive	Elton (1959)
	Rbt	Carb. An.	SC	Inactive	Elton (1959)
	Rbt	Carb. An.	O	Inactive	Elton (1959)
	Rbt	McG.	IU	Inactive	Elton (1959)
Pregn-4-ene-3,20-dione, 21-fluoro-17α-hydroxy-, acetate	Rbt	Cl.	SC	10 × P	Bergstrom *et al.* (1960), Elton (1959)
	Rbt	Cl.	O	1 × P (SC)	Bergstrom *et al.* (1960), Elton (1959)
	Rbt	McG.	IU	1 × P	Elton (1959)
Pregn-4-ene-3,20-dione, 21-fluoro-17α-hydroxy-, caproate	Rbt	Cl.	SC	0.5 × P	Bergstrom *et al.* (1960)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Pregn-4-ene-3,20-dione, 21-fluoro-17α-hydroxy-6α-methyl-, acetate	Rbt	Cl.	SC	50 × P	Bergstrom *et al.* (1960), Elton (1959)
	Rbt	Cl.	O	10 × P	Bergstrom *et al.* (1960), Elton (1959)
	Rbt	Carb. An.	SC	40–50 × P	Elton (1959), Elton *et al.* (1960)
	Rbt	Carb. An.	O	10 and 17 × P	Elton (1959), Elton *et al.* (1960)
	Rbt	McG.	IU	50 × P	Elton (1959), Elton *et al.* (1960)
Pregn-4-ene-3,20-dione, 21-fluoro-6α,17α-dimethyl-	Rbt	Cl.	SC	10 × P	Deghenghi *et al.* (1963a)
	Rbt	Cl.	O	< 1 × ENT	Deghenghi *et al.* (1963a)
Pregn-4-ene-3,20-dione, 21-fluoro-17α-methyl-	Rbt	Cl.	SC	> 10 × P	Deghenghi *et al.* (1963a)
	Rbt	Cl.	O	< 0.1 × ENT	Deghenghi *et al.* (1963a)
Pregn-4-ene-3,20-dione, 21,21-difluoro-	—	—	SC	Inactive	Nakanishi *et al.* (1959)
	Rbt	Cl.	—	Weak activity	Edwards and Ringold, (1959)
Pregn-4-ene-3,20-dione, 21,21,21-trifluoro-	—	—	SC	Inactive	Nakanishi *et al.* (1959)
Pregn-4-ene-3,20-dione, 11β-formyloxy-	Rbt	Cl.	SC	Inactive	Oliveto *et al.* (1958)
Pregn-4-ene-3,20-dione, 11β,17α-diformyloxy-	Rbt	Cl.	SC	0.08 × P	Oliveto *et al.* (1958)
Pregn-4-ene-3,20-dione, 17α-formyloxy-11β-hydroxy-, acetate	Rbt	Cl.	SC	Inactive	Oliveto *et al.* (1958)
Pregn-4-ene-3,20-dione, 2α-hydroxy-, acetate	Rbt	Carb. An.	—	Inactive	Lutwak-Mann and Adams (1957)
Pregn-4-ene-3,20-dione, 2β-hydroxy-, acetate	Rbt	Carb. An.	—	Inactive	Lutwak-Mann and Adams (1957)
Pregn-4-ene-3,20-dione, 4,17α-dihydroxy-, 17-acetate	Rbt	Cl.	SC	4.73 × P	Arcari *et al.* (1963)
	Rbt	Cl.	O	5.50 × AP	Arcari *et al.* (1963)

	Rbt	Carb. An.	—	Active	Arcari *et al.* (1963)
	Rbt	McG.	IU	Active	Arcari *et al.* (1963)
	R	Decid.	—	1 × P	Arcari *et al.* (1963)
	M	P-M	—	Active	Arcari *et al.* (1963)
Pregn-4-ene-3,20-dione, 6-hydroxy-	Rbt	McG.	IU	Markedly < P	Salhanick and Swanson (1960)
Pregn-4-ene-3,20-dione, 6β-hydroxy-	Rbt	McG.	IU	Inactive	Salhanick and Swanson (1961)
	M	H-F	IU	0.0003 × P	Zarrow *et al.* (1957)
Pregn-4-ene-3,20-dione, 6β-hydroxy-, acetate	Rbt	McG.	IU	Inactive	Salhanick and Swanson (1961)
	Rbt	Cl.	SC	0.3–0.2 × P	Meystre *et al.* (1948)
	Rbt	C-A	SC	0.3–0.2 × P	Meystre *et al.* (1948)
Pregn-4-ene-3,20-dione, 6β,11α-dihydroxy-	M	H-F	IU	0.002 × P	Zarrow *et al.* (1957)
Pregn-4-ene-3,20-dione, 9-hydroxy-	Rbt	McG.	IU	Markedly < P	Salhanick and Swanson (1960)
Pregn-4-ene-3,20-dione, 9α-hydroxy-	Rbt	McG.	IU	Inactive	Salhanick and Swanson (1961)
Pregn-4-ene-3,20-dione, 11-hydroxy-	Rbt	McG.	IU	Markedly < P	Salhanick and Swanson (1960)
Pregn-4-ene-3,20-dione, 11α-hydroxy-	Rbt	McG.	IU	Inactive	Salhanick and Swanson (1961)
	—	—	—	0.03–0.1 × P	Mardones *et al.* (1954a)
Pregn-4-ene-3,20-dione, 11α-hydroxy-, acetate	Rbt	McG.	IU	Inactive	Salhanick and Swanson (1961)
Pregn-4-ene-3,20-dione, 11β-hydroxy-	Rbt	Cl.	—	Inactive	Byrnes and Shipley (1955)
	Rbt	McG.	IU	Inactive	Salhanick and Swanson (1961)
	M	H-F	IU	0.015 × P	Zarrow *et al.* (1957)
	—	—	—	0.03–0.1 × P	Mardones *et al.* (1954a)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Pregn-4-ene-3,20-dione, 11β-hydroxy-, acetate	Rbt	Cl.	SC	Inactive	Oliveto *et al* (1958)
	Rbt	McG.	IU	Inactive	Salhanick and Swanson (1961)
Pregn-4-ene-3,20-dione, 11α,17α-dihydroxy-	M	H-F	IU	0.003 × P	Zarrow *et al.* (1957)
Pregn-4-ene-3,20-dione, 11β,17α-dihydroxy-, 11-acetate	Rbt	Cl.	SC	Inactive	Oliveto *et al.* (1958)
Pregn-4-ene-3,20-dione, 11β,17α-dihydroxy-, diacetate	Rbt	Cl.	SC	0.2 × P	Oliveto *et al.* (1958)
Pregn-4-ene-3,20-dione, 11β,17α-dihydroxy-, 11-acetate 17-caproate	Rbt	Cl.	SC	0.08 × P	Oliveto *et al.* (1958)
Pregn-4-ene-3,20-dione, 11β,17α-21-trihydroxy-, 21-acetate	Rbt	Cl.	SC	Inactive	Hertz and Tullner (1956)
Pregn-4-ene-3,20-dione, 11β,21-dihydroxy-, 21-acetate	Rbt	Cl.	SC	0.14–0.25 ×	Engel and Noble (1956)
Pregn-4-ene-3,20-dione, 12α-hydroxy-	Rbt	Cl.	SC	< 0.06 × P	Meystre *et al.* (1948)
Pregn-4-ene-3,20-dione, 14-hydroxy-	Rbt	McG.	IU	Markedly < P	Salhanick and Swanson (1960)
Pregn-4-ene-3,20-dione, 14α-hydroxy-	Rbt	McG.	IU	Inactive	Salhanick and Swanson (1961)
Pregn-4-ene-3,20-dione, 15-hydroxy-	Rbt	McG.	IU	Markedly < P	Salhanick and Swanson (1960)
Pregn-4-ene-3,20-dione, 15α-hydroxy-	Rbt	McG.	IU	Inactive	Salhanick and Swanson (1961)
Pregn-4-ene-3,20-dione, 15α-hydroxy-, acetate	Rbt	McG.	IU	Inactive	Salhanick and Swanson (1961)
Pregn-4-ene-3,20-dione, 16-hydroxy-	Rbt	McG.	IU	Markedly < P	Salhanick and Swanson (1960)

Pregn-4-ene-3,20-dione, 16α-hydroxy-	Rbt	McG.	IU	Inactive	Salhanick and Swanson (1961)
Pregn-4-ene-3,20-dione, 16α-hydroxy-, acetate	Rbt	McG.	IU	Inactive	Salhanick and Swanson (1961)
Pregn-4-ene-3,20-dione, 16α,17α-dihydroxy-	Rbt	Cl.	O	Inactive	Rapala and Kraay (1962), Lerner *et al.* (1961a)
	Rbt	Cl.	SC	Inactive	Lerner *et al.* (1961a)
Pregn-4-ene-3,20-dione, 16α,17α-dihydroxy-,	Rbt	Cl.	SC	32–64 × P	Lerner *et al.* (1963)
2-acetofuran	Rbt	Cl.	O	0.13–0.25 × ENT	Lerner *et al.* (1963)
	R	P-M	—	> P	Lerner *et al.* (1963)
Pregn-4-ene-3,20-dione, 16α,17α-dihydroxy-,	Rbt	Cl.	SC	1 × P	Lerner *et al.* (1963)
acetophenone	Rbt	Cl.	O	1 × ENT	Lerner *et al.* (1963)
Pregn-4-ene-3,20-dione, 17α-hydroxy-	Rbt	Cl.	SC	Inactive	Elton (1959), Pfiffner and North (1940)
	Rbt	Cl.	SC	< P, 0.01 × P	Junkmann (1954), Kessler and Borman (1958)
	Rbt	Cl.	IM	Inactive	Pfiffner and North (1941)
	Rbt	Cl.	O	Inactive, 0.001 × ENT	Elton (1959), Kincl and Folch-Pi (1962a)
	Rbt	McG.	IU	Inactive	Salhanick *et al.* (1957) Salhanick and Swanson (1960, 1961), Elton (1959)
	Rbt	Carb. An.	SC	Inactive	Lutwak-Mann and Adams (1957), Elton (1959)
	Rbt	Carb. An.	O	Inactive	Elton (1959)
	Rbt	C-A	SC	Inactive	Byrnes (1960)
	Rbt	C-A	O	Inactive	Byrnes (1960)
	Rbt	C-A	IM	Inactive	Salhanick *et al.* (1957)
	M	H-F	IU	60 × P	Salhanick *et al.* (1957), Zarrow *et al.* (1957)
	M	H-F	IU	Many × P	Byrnes (1960)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Pregn-4-ene-3,20-dione, 17α-hydroxy-	M	H-F	IU	±	Nakao *et al.* (1958)
	R	Decid.	—	$< 0.02 \times P$	Zarrow *et al.* (1958)
	M	Decid.	—	$< 0.04 \times P$	Zarrow *et al.* (1958)
	Chick	Oviduct	—	$0.15 \times P$	Zarrow *et al.* (1958)
Pregn-4-ene-3,20-dione, 17α-hydroxy-, acetate	Rbt	Cl.	SC	$> P$	Junkmann (1954)
	Rbt	Cl.	—	$< P$	Wada (1959)
	Rbt	Cl.	SC	$2–6 \times P$	Babcock *et al.* (1958), Barnes *et al.* (1959), Deghenghi *et al.* (1963b), Kincl and Folch-Pi (1962b)
	Rbt	Cl.	SC	$10 \times P$	Bergstrom *et al.* (1960), Elton (1959)
	Rbt	Cl.	O	$0.2 \times P$ (SC)	Bergstrom *et al.* (1960), Elton (1959)
	Rbt	Cl.	O	$0.07–0.15 \times ENT$	Deghenghi *et al.* (1963b), Kincl (1961), Kincl and Folch-Pi (1962a), Ringold *et al.* (1959a)
	Rbt	Cl.	O	$2–6 \times ET$	Barnes *et al.* (1959), Babcock *et al.* (1958)
	Rbt	McG.	IU	Sl. $< P$	Salhanick and Swanson (1960)
	Rbt	McG.	IU	Mod. activity $> P$	Salhanick and Swanson (1961)
	Rbt	McG.	IU	$1 \times P$	Elton (1959), Elton *et al.* (1960)
	Rbt	Carb. An.	SC	$7–10 \times P$	Barnes *et al.* (1959), Elton (1959), Elton *et al.* (1960),

					Miyake and Pincus (1958)
	Rbt	Carb. An.	O	$0.2 \times P$	Elton (1959), Elton *et al.* (1960)
	Rbt	GMR	SC	$3.3 \times P$	Barnes *et al.* (1959), Miyake and Pincus (1958)
	Rbt	C-A	—	Active	Wada (1959)
	Rbt	C-A	O	Active	Byrnes (1960)
	Rbt	P-M	O	Inactive	Falconi *et al.* (1961)
	R	P-M	SC	Active ($<$P)	Stucki (1958)
	R	P-M	SC	Inactive	Saunders and Elton (1959)
	R	Estrus	O	Inactive	Falconi and Ercoli (1961)
Pregn-4-ene-3,20-dione, 17α-hydroxy-, β-(*p*-butoxyphenyl) propionate	Rbt	Cl.	IM	Prolonged activity	Diczfalusy (1960), Diczfalusy *et al.* (1961)
Pregn-4-ene-3,20-dione, 17α-hydroxy-, *n*-butyrate	Rbt	Cl.	SC	$>$P	Junkmann (1954)
Pregn-4-ene-3,20-dione, 17α-hydroxy-, *n*-caprinate	Rbt	Cl.	SC	$<$P	Junkmann (1954)
Pregna-4-ene-3,20-dione, 17α-hydroxy-, caproate	Rbt	Cl.	—	$<$P	Wada (1959)
	Rbt	Cl.	SC	$2 \times P$, long acting	Kessler and Borman (1957, 1958)
	Rbt	Cl.	SC	Very potent, long acting ($>$P)	Junkmann (1954)
	Rbt	Cl.	IM	Long acting	Diczfalusy (1960)
	Rbt	Carb. An.	—	Active	Lutwak-Mann and Adams (1957)
	Rbt	C-A	—	Active	Wada (1959)
	Rbt	P-M	—	Active	Lutwak-Mann and Adams (1957)
	M	P-M	—	Inactive	Smithberg (1958)
	R	P-M	SC	Inactive	Saunders and Elton (1959)
	R	P-M	O	Inactive	Saunders and Elton (1959)
	R	Decid.	—	$<0.02 \times P$	Zarrow *et al.* (1958)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Pregna-4-ene-3,20-dione, 17α-hydroxy-, caproate	M	Decid.	—	< 0.04 × P	Zarrow *et al.* (1958)
	Chick	Oviduct	—	0.08 × P	Zarrow *et al.* (1958)
	Rats	Estrus	—	Inactive	Velardo (1958)
Pregn-4-ene-3,20-dione, 17α-hydroxy-, *n*-caprylate	Rbt	Cl.	SC	< P	Junkmann (1954)
Pregn-4-ene-3,20-dione, 17α-hydroxy-, diethylacetate	Rbt	Cl.	SC	1 × P	Junkmann (1954)
Pregn-4-ene-3,20-dione, 17α-hydroxy-, *n*-enanthate	Rbt	Cl.	SC	> P	Junkmann (1954)
	Rbt	Cl.	SC	0.05 × P	Gleason and Parker (1959)
Pregn-4-ene-3,20-dione, 17α-hydroxy-, β-(*p*-hexoxyphenyl) propionate	Rbt	Cl.	IM	Prolonged activity	Diczfalusy *et al.* (1961)
Pregn-4-ene-3,20-dione, 17α-hydroxy-, β-(*p*-octoxyphenyl) propionate	Rbt	Cl.	IM	Prolonged activity	Diczfalusy *et al.* (1961)
Pregn-4-ene-3,20-dione, 17α-hydroxy-, β-(*p*-pentoxyphenyl) propionate	Rbt	Cl.	IM	Prolonged activity	Diczfalusy *et al.* (1961)
Pregn-4-ene-3,20-dione, 17α-hydroxy-, propionate	Rbt	Cl.	SC	1 × P	Junkmann (1954)
Pregn-4-ene-3,20-dione, 17α-hydroxy-, *n*-undecanoate	Rbt	Cl.	SC	< P	Junkmann (1954)
Pregn-4-ene-3,20-dione, 17α-hydroxy-, *n*-valerate	Rbt	Cl.	SC	> P	Junkmann (1954)
Pregn-4-ene-3,20-dione, 17α-hydroxy-21-iodo-, acetate	Rbt	Cl.	SC	Inactive	Bergstrom *et al.* (1960), Elton (1959)
	Rbt	McG.	IU	0.5–1.0 × P	Elton (1959)
Pregn-4-ene-3,20-dione, 17α-hydroxy-6α-methyl-, acetate	Rbt	Cl.	SC	10–20 × P	Camerino *et al.* (1962), Deghenghi *et al.* (1963b), Sala *et al.* (1958)
	Rbt	Cl.	SC	50–60 × P	Babcock *et al.* (1958), Bergstrom *et al.* (1960), Elton (1959)

Rbt	Cl.	O	3–5 × P (SC)	Bergström *et al.* (1960), Camerino *et al.* (1962), Elton (1959)
Rbt	Cl.	O	Potent	Andreoli (1962)
Rbt	Cl.	O	2–3 × ENT	Madjerek *et al.* (1960), Kincl (1961), Ringold *et al.* (1959a,b)
Rbt	Cl.	O	8.3 × P (SC)	Sala *et al.* (1958)
Rbt	Cl.	O	100–300 × ET	Babcock *et al.* (1958), Sala *et al.* (1958)
Rbt	Cl.	O	10 × ENT	Deghenghi *et al.* (1963b)
Rbt	Cl.	O	40–50 × AP	Arcari *et al.* (1963), Camerino *et al.* (1962)
Rbt	McG.	IU	High activity (> P)	Salhanick and Swanson (1961)
Rbt	McG.	IU	25 × P	Elton (1959), Elton *et al.* (1960)
Rbt	Carb. An.	SC	10–15 × P	Sala *et al.* (1958), Camerino *et al.* (1962)
Rbt	Carb. An.	SC	30–44 × P	Elton (1959), Elton *et al.* (1960), Miyake and Pincus (1958)
Rbt	Carb. An.	O	4–8 × P (SC)	Camerino *et al.* (1962), Elton (1959), Elton *et al.* (1960), Sala *et al.* (1958)
Rbt	Carb. An.	O	13.6 × AP	Miyake and Pincus (1958)
Rbt	GMR	SC	33.8 × P	Miyake and Pincus (1958)
Rbt	GMR	O	13.0 × AP	Miyake and Pincus (1958)
Rbt	C-A	SC	20 × P	Wu (1961)
Rbt	C-A	O	0.25 × P (SC)	Wu (1961)
—	P-M	—	Active	Wu (1961)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Pregn-4-ene-3,20-dione-, 17α-hydroxy-6α-methyl-acetate	Rbt	P-M	O	1 × P (SC)	Falconi *et al.* (1961)
	Rbt	P-M	SC	25–100 × P	Babcock *et al.* (1958)
	R	P-M	SC	Active (> P)	Stucki (1958)
	R	P-M	O	Active	Suchowsky (1962)
	R	P-M	—	Poor	Madjerek *et al.* (1960)
	—	P-M	O	Mod. activity	Andreoli (1962)
	—	Decid.	O	Mod. activity	Andreoli (1962)
	M	Decid.	—	Failed	Madjerek *et al.* (1960)
	R	Estrus	O	Active	Falconi and Ercoli (1961)
Pregn-4-ene-3,20-dione, 17α-hydroxy-6-methyl-, acetate	R	P-M	SC	25 × P	Saunders and Elton (1959)
	Rbt	P-M	SC	> P	Saunders and Elton (1959)
Pregn-4-ene-3,20-dione, 17α-hydroxy-6β-methyl-, acetate	Rbt	McG.	IU	5 × P	Elton *et al.* (1960)
	Rbt	Carb. An.	SC	20 × P	Elton *et al.* (1960)
	Rbt	Carb. An.	O	2 × P	Elton *et al.* (1960)
Pregn-4-ene-3,20-dione, 17α-hydroxy-6α,16-dimethyl-, acetate	Rbt	Cl.	—	100 × 6α,21-dimethyl ET	Petrow and Williamson (1962)
Pregn-4-ene-3,20-dione, 17α-hydroxy-6α,16α-dimethyl-, acetate	Rbt	Cl.	O	2–4 × ENT	Kincl and Folch-Pi (1962a)
Pregn-4-ene-3,20-dione, 17α-hydroxy-16α-methyl-	Rbt	Cl.	O	Inactive	Bernstein *et al.* (1961a)
Pregn-4-ene-3,20-dione, 17α-hydroxy-16α-methyl-, acetate	Rbt	Cl.	O	1 × AP	Bernstein *et al.* (1961a)
	Rbt	Cl.	O	0.1 × ENT	Kincl and Folch-Pi (1962a)
Pregn-4-ene-3,20-dione, 17α-hydroxy-16β-methyl-, acetate	Rbt	Cl.	—	1 × P	Sciaky (1961)
Pregn-4-ene-3,20-dione, 17α-hydroxy-16-methylene-, acetate	—	—	—	> AP	Kirk *et al.* (1961)
Pregn-4-ene-3,20-dione, 17α-hydroxy-6α-nitro-, acetate	Rbt	Cl.	O	3–4 × AP	Bowers *et al.* (1959a)

Pregn-4-ene-3,20-dione, 17α,21-dihydroxy-, diacetate	Rbt	Cl.	O	Low activity	Ringold *et al.* (1959b)
Pregn-4-ene-3,20-dione, 17α,21-dihydroxy-6α-methyl-, diacetate	Rbt	Cl.	O	1.5 × ENT	Ringold *et al.* (1959b)
	Rbt	Cl.	O	32 × DAP	Kincl (1962)
Pregn-4-ene-3,20-dione, 18-hydroxy-	Rbt	McG.	IU	Inactive	Salhanick and Swanson (1960, 1961)
Pregn-4-ene-3,20-dione, 18-hydroxy-, acetate	Rbt	McG.	IU	Inactive	Salhanick and Swanson (1961)
Pregn-4-ene-3,20-dione, 19-hydroxy-	Rbt	Cl.	SC	< 0.1 × P	Barber and Ehrenstein (1954a,b)
	Rbt	C-A	SC	< 0.1 × P	
	Rbt	McG.	IU	Inactive	Salhanick and Swanson (1960, 1961)
Pregn-4-ene-3,20-dione, 19-hydroxy-, acetate	Rbt	McG.	IU	Inactive	Salhanick and Swanson (1961)
Pregn-4-ene-3,20-dione, 21-hydroxy-	Rbt	Cl.	SC	0.1–0.15 × P	Engel and Noble (1957), Meystre *et al.* (1948)
	Rbt	Cl.	IM	0.1–0.25 × P	Diczfalusy (1960), Pfiffner and North (1941)
	Rbt	C-A	SC	0.1 × P	Meystre *et al.* (1948)
	Rbt	McG.	IU	Mod. activity (< P)	Salhanick and Swanson (1960)
	Rbt	Carb. An.	—	Inactive	Lutwak-Mann and Adams (1957)
	M	H-F	IU	Active	Nakao *et al.* (1958)
Pregn-4-ene-3,20-dione, 21-hydroxy-, acetate	Rbt	Cl.	SC	0.03 × P	Hertz and Tullner (1956)
	Rbt	Carb. An.	SC	Active	Adams and Lutwak-Mann (1955)
	Rbt	Carb. An.	—	Weak activity	Lutwak-Mann and Adams (1957)
	Rbt	McG.	IU	Sl > P	Salhanick and Swanson (1960)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Pregn-4-ene-3,20-dione, 21-hydroxy-, acetate	M	H-F	IU	Inactive	Hooker and Forbes (1947)
	R	P-M	SC	Active	Stucki (1958)
Pregn-4-ene-3,20-dione, 21-hydroxy-, *p*-hexoxyphenylpropionate	Rbt	Cl.	IM	Long acting	Diczfalusy (1960)
Pregn-4-ene-3,20-dione, 21-hydroxy-17α-methyl-, acetate	Rbt	Cl.	SC	$1 \times P$	Engel and Noble (1956)
Pregn-4-ene-3,20-dione, 19:8-lacto-	Rbt	Cl.	—	$< 0.1 \times P$	Barber and Ehrenstein (1961)
Pregn-4-ene-3,20-dione, 2α-methyl-	Rbt	McG.	IU	Sl. active ($<$P)	Salhanick and Swanson (1960, 1961)
Pregn-4-ene-3,20-dione, 2β-methyl-	Rbt	McG.	IU	Inactive	Salhanick and Swanson (1960, 1961)
Pregn-4-ene-3,20-dione, 2α,16α-dimethyl-	Rbt	Cl.	SC	Inactive	Bernstein *et al.* (1961b)
Pregn-4-ene-3,20-dione, 4-methyl-	Rbt	Cl.	SC	$0.5 \times P$	Sondheimer and Mazur (1957)
Pregn-4-ene-3,20-dione, 6α-methyl-	Rbt	Cl.	SC	$5 \times P$	Elton (1959)
	Rbt	Cl.	O	$0.2 \times P$	Elton (1959)
	Rbt	Cl.	O	$< 0.1 \times ENT$	Deghenghi *et al.* (1963b)
	Rbt	McG	IU	$1 \times P$	Salhanick and Swanson (1961)
	Rbt	McG.	IU	$5 \times P$	Elton (1959), Elton *et al.* (1960)
	Rbt	Carb. An.	SC	$5 \times P$	Elton (1959), Elton *et al.* (1960)
	Rbt	Carb. An.	O	$0.2 \times P$	Elton (1959), Elton *et al.* (1960)
Pregn-4-ene-3,20-dione, 6β-methyl-	Rbt	McG.	IU	$1 \times P$	Salhanick and Swanson (1961)

Pregn-4-ene-3,20-dione, 6α-methyl-17α-hydroxy-, acetate	Rbt	Cl.	SC	6–8 × AP	Barnes *et al.* (1959)
	Rbt	Cl.	O	60–75 × AP	Barnes *et al.* (1959)
Pregn-4-ene-3,20-dione, 6α-methyl-16α,17α-isopropylidenedioxy-	—	—	—	Active	Bianchi *et al.* (1961)
	Rbt	Cl.	SC	1–2 × P	Lerner *et al.* (1961a)
	Rbt	Cl.	O	0.625 × ENT	Lerner *et al.* (1961a)
Pregn-4-ene-3,20-dione, 6β-methyl-16α,17α-isopropylidenedioxy-	Rbt	Cl.	SC	1–2 × P	Lerner *et al.* (1961a)
	Rbt	Cl.	O	0.625 × ENT	Lerner *et al.* (1961a)
Pregn-4-ene-3,20-dione, 6α,16α-dimethyl-	Rbt	Cl.	SC	1 × P	Bernstein *et al.* (1961a,b)
	Rbt	Cl.	SC	2.25 × P	Kincl and Folch-Pi (1962b)
Pregn-4-ene-3,20-dione, 6β,16α-dimethyl-	Rbt	Cl.	SC	4 × P	Kincl and Folch-Pi (1962b)
	Rbt	Cl.	O	0.4 × P	Kincl and Folch-Pi (1962a)
Pregn-4-ene-3,20-dione, 6α,17α-dimethyl-	Rbt	Cl.	SC	5 × P	Deghenghi *et al.* (1963a,b)
	Rbt	Cl.	O	1 × ENT	Deghenghi *et al.* (1963a,b)
	Rbt	Cl.	O	20 × methyl-P	Deghenghi and Gaudry (1961)
Pregn-4-ene-3,20-dione, 7α-methyl-	Rbt	McG.	IU	1 × P	Salhanick and Swanson (1961)
Pregn-4-ene-3,20-dione, 7β-methyl-	Rbt	McG.	IU	1 × P	Salhanick and Swanson (1961)
Pregn-4-ene-3,20-dione, 16α-methyl-	Rbt	Cl.	SC	1 × P	Bernstein *et al.* (1961b)
	Rbt	McG.	IU	High activity (> P)	Salhanick and Swanson (1961)
Pregn-4-ene-3,20-dione, 17α-methyl-	—	—	—	3 × P	Deghenghi (1962)
	Rbt	Cl.	SC	2 × P	Deghenghi *et al.* (1963a,b)
	Rbt	Cl.	O	0.05 × ENT	Deghenghi *et al.* (1963a,b)
	Rbt	C-A	—	2 × P	Heusser *et al.* (1950a)
Pregn-4-ene-3,20-dione, 21-methyl-	Rbt	Cl.	SC	0.15 × P	Meystre *et al.* (1948)
	Rbt	Cl.	SC	Active	Wettstein (1940b)
	Rbt	Cl.	O	Inactive	Wettstein (1940b)
	Rbt	C-A	SC	0.15 × P	Meystre *et al.* (1948)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Pregn-4-ene-3,20-dione, 16α-nitromethyl-	Rbt	Cl.	SC	$<0.05 \times P$	Drill and Riegel (1958)
	Rbt	McG.	IU	$<0.005 \times P$	Drill and Riegel (1958)
Pregn-4-ene-3,20-dione, 17α-propyl-	Rbt	Cl.	SC	$2 \times P$	Deghenghi *et al.* (1963b)
	Rbt	Cl.	O	$0.1 \times ENT$	Deghenghi *et al.* (1963b)
Pregn-4-ene-3,20-dione, 16α,17α-isopropylidenedioxy-	Rbt	Cl.	SC	$1–2 \times P$	Lerner *et al.* (1961a)
	Rbt	Cl.	O	$0.125 \times ENT$	Lerner *et al.* (1961a)
Pregn-4-en-20-one	Rbt	Cl.	SC	$<0.08 \times P$	Kincl and Folch-Pi (1962b)
Pregn-4-en-20-one, 3-benziloylhydrazone-17α-hydroxy-, enanthate	Rbt	Cl.	SC	$0.1 \times P$	Gleason and Parker (1959)
Pregn-4-en-20-one, 3β-hydroxy-	—	—	—	$1 \times P$	Gut (1956)
Pregn-4-en-20-one, 17α-hydroxy-, acetate	Rbt	Cl.	O	$0.15 \times ENT$	Kincl and Folch-Pi (1962a)
Pregn-4-en-20-one, 3-oxime	Rbt	Cl.	SC	$<P$	Junkmann (1954)
Pregn-4-en-20-one, 3-*N*-phenylimino-	Rbt	Cl.	SC	$>P$	Junkmann (1954)
Pregn-4-en-20-one, 3-monopropylene ketal	Rbt	Cl.	—	$<P$	Wada (1959)
9β,10α-Pregna-4,6-diene-3,20-dione	Rbt	Cl	SC	$25–34 \times P$	Reerink *et al.* (1960), Schöler (1960)
	Rbt	Cl.	O	High activity	Reerink *et al.* (1960)
	Rbt	Cl.	O	$0.4 \times P$ (SC)	Schöler (1960)
9β,10α-Pregna-4,6-diene-3,20-dione, 17α-hydroxy-, acetate	Rbt	Cl.	SC	$78.7 \times P$	Schöler (1960)
	Rbt	Cl.	O	$6 \times P$ (SC)	Schöler (1960)
	Rbt	Cl.	O	$<0.3 \times AP$	Arcari *et al.* (1963)
10α-Pregna-4,6-diene-3,20-dione	Rbt	Cl.	O	$0.3 \times ENT$	Kincl and Folch-Pi (1962a)
Pregna-4,6-diene-3,20-dione	Rbt	Cl.	—	Active	Hegner and Reichstein (1943)
	Rbt	Cl.	SC	$0.4–0.5 \times P$	Meystre *et al.* (1948)
	Rbt	C-A	SC	$0.4–0.5 \times P$	Meystre *et al.* (1948), Wettstein (1940a)
	Rbt	C-A	O	Inactive	Wettstein (1940a)

	Rbt	C-A	—	1 × P	Shoppee and Reichstein (1941)
	Rbt	McG.	IU	Mod. activity (< P)	Salhanick and Swanson (1961)
	M	H-F	IU	0.015 × P	Zarrow *et al.* (1957)
Pregna-4,6-diene-3,20-dione, 17α-acetyl-6-methyl-	Rbt	Cl.	O	> 100 × 6α,21-dimethyl-ET	Hartley (1962)
	R	P-M	O	Active	Hartley (1962)
Pregna-4,6-diene-3,20-dione, 17α-acetyl-6-methyl-16-methylene-	Rbt	Cl.	O	> 100 × 6α,21-dimethyl-ET	Hartley (1962)
	R	P-M	O	Active	Hartley (1962)
Pregna-4,6-diene-3,20-dione, 17α-bromo-6-methyl-	Rbt	Cl.	SC	1 × P	Deghenghi *et al.* (1963b)
	Rbt	Cl.	O	1 × ENT	Deghenghi *et al.* (1963b)
Pregna-4,6-diene-3,20-dione, 6-chloro-17α-ethyl-	Rbt	Cl.	SC	10 × P	Deghenghi *et al.* (1963b)
	Rbt	Cl.	O	20 × ENT	Deghenghi *et al.* (1963b)
Pregna-4,6-diene-3,20-dione, 6-chloro-21-fluoro-17α-methyl-	Rbt	Cl.	SC	5 × P	Deghenghi *et al.* (1963a)
	Rbt	Cl.	O	10 × ENT	Deghenghi (1963a)
Pregna-4,6-diene-3,20-dione, 6-chloro-17α-hydroxy-, acetate	Rbt	Cl.	SC	50 × P	Kincl and Dorfman (1961)
	Rbt	Cl.	SC	6–10 × AP	Kincl (1961)
	Rbt	Cl.	O	35–50 × ENT	Ringold *et al.* (1959a), Zderic (1963), Kincl (1961)
	Rbt	McG.	IU	1 × P	Salhanick and Swanson (1961)
Pregna-4,6-diene-3,20-dione, 6-chloro-17α-hydroxy-1,2α-methylene-, acetate	R	P-M	—	Inactive	Suchowsky (1962)
Pregna-4,6-diene-3,20-dione, 6-chloro-17α,21-dihydroxy-, diacetate	Rbt	Cl.	O	1.5 × ENT	Ringold *et al.* (1959a)
	Rbt	Cl.	O	32 × DAP	Kincl (1962)
Pregna-4,6-diene-3,20-dione, 6-chloro-17α-methyl-	Rbt	Cl.	SC	5–8 × P	Deghenghi *et al.* (1963a)
	Rbt	Cl.	O	20 × ENT	Deghenghi *et al.* (1963a)
Pregna-4,6-diene-3,20-dione, 17α-ethyl-6-methyl-	Rbt	Cl.	SC	5 × P	Deghenghi *et al.* (1963b)
	Rbt	Cl.	O	20 × ENT	Deghenghi *et al.* (1963b)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Pregna-4,6-diene-3,20-dione, 6-fluoro-17α-hydroxy-, acetate	Rbt	Cl.	O	15–18 × ENT	Kincl (1961), Ringold *et al.* (1959a), Bowers *et al.* (1959b)
	Rbt	McG.	IU	Sl. activity (< P)	Salhanick and Swanson (1961)
Pregna-4,6-diene-3,20-dione, 21-fluoro-17α-hydroxy-6-methyl-, acetate	Rbt	Cl.	O	17 × P (SC)	Sollman *et al.* (1959)
	Rbt	McG.	IU	50 × P	Elton *et al.* (1960)
	Rbt	Carb. An.	SC	40 × P	Elton *et al.* (1960)
	Rbt	Carb. An.	O	20 × P	Elton *et al.* (1960)
Pregna-4,6-diene-3.20-dione, 21-fluoro-6,17α-dimethyl-	Rbt	Cl.	SC	10 × P	Deghenghi *et al.* (1963a)
	Rbt	Cl.	O	< 10 × ENT	Deghenghi *et al.* (1963a)
Pregna-4,6-diene-3,20-dione, 17α-hydroxy-, acetate	Rbt	Cl.	SC	5.5 × P	Kincl and Folch-Pi (1962b)
	Rbt	Cl.	SC	< AP	Kincl (1961)
	Rbt	Cl.	O	1.5 × ENT	Kincl (1961)
Pregna-4,6-diene-3,20-dione, 17α-hydroxy-6-methyl-, acetate	—	—	O	Potent	Barton *et al.* (1962)
	Rbt	Cl.	O	12–15 × ENT	Ringold *et al.* (1959a,b), Kincl (1961)
	Rbt	McG.	IU	High activity (> P)	Salhanick and Swanson (1961)
	Rbt	McG.	IU	10 × P	Elton *et al.* (1960)
	Rbt	Carb An.	SC	25 × P	Elton *et al.* (1960)
	Rbt	Carb. An.	O	17 × P	Elton *et al.* (1960)
Pregna-4,6-diene-3,20-dione, 17α-hydroxy-6,16α-dimethyl-, acetate	Rbt	Cl.	O	Potent > 6α,21-dimethyl ET	Barton *et al.* (1962)
Pregna-4,6-diene-3,20-dione, 17α-hydroxy-16α-methyl-, acetate	Rbt	Cl.	O	15 × AP	Bernstein *et al.* (1961b)
Pregna-4,6-diene-3,20-dione, 6-methyl-	Rbt	Cl.	O	< 1 × ENT	Deghenghi *et al.* (1963b)
	Rbt	McG.	IU	0.5 × P	Elton *et al.* (1960)
	Rbt	Carb. An.	SC	2 × P	Elton *et al.* (1960)

Pregna-4,6-diene-3,20-dione, 6,16α-dimethyl-	Rbt	Cl.	O	0.2 × ENT	Kincl and Folch-Pi (1962a)
Pregna-4,6-diene-3,20-dione, 6,17α-dimethyl-	Rbt	Cl.	SC	0.35 × P	Kincl and Folch-Pi (1962b)
	Rbt	Cl.	SC	5 × P	Deghenghi *et al.* (1963a,b)
	Rbt	Cl.	O	10 × ENT	Deghenghi *et al.* (1963a,b)
	Rbt	Cl.	O	20 × methyl-P	Deghenghi and Gaudry (1961)
Pregna-4,6,11-triene-3,20-dione, 17α-hydroxy-, acetate	Rbt	Cl.	O	7 × AP	Dusza *et al.* (1963)
Pregna-4,9(11)-diene-3,20-dione	Rbt	Cl.	SC	0.25–0.5 × P	Meystre *et al.* (1948)
	Rbt	C-A	SC	0.25–0.5 × P	Meystre *et al.* (1948)
	Rbt	Cl.	—	Active	Hegner and Reichstein (1943)
Pregna-4,9(11)-diene-3,20-dione, 21-fluoro-17α-hydroxy-	—	—	SC	10–20 × P	Pederson and Babcock (1962)
	—	—	O	Active	Pederson and Babcock (1962)
Pregna-4,9(11)-diene-3,20-dione, 21-fluoro-17α-hydroxy-, propionate	—	—	SC	10–20 × P	Pederson and Babcock (1962)
	—	—	O	Active	Pederson and Babcock (1962)
Pregna-4,11-diene-3,20-dione	—	—	—	3 × P	Mardones *et al.* (1954b)
	Rbt	Cl.	—	0.5 × P	Hegner and Reichstein (1943), Meystre *et al.* (1948)
	Rbt	Cl.	SC	2–3 × P	Meystre *et al.* (1948), Zarrow *et al.* (1958)
	Rbt	McG.	IU	High activity (> P)	Salhanick and Swanson (1960, 1961)
	Rbt	C-A	SC	0.5 × P	Meystre *et al.* (1948), Shoppee and Reichstein (1941)

continued

TABLE XI—*continued*

Compound	Animal	Test	Route	Response	References
Pregna-4,11-diene-3,20-dione	Rbt	C-A	—	> Methyl-P	Heusser *et al.* (1950a)
	M	H-F	IU	1.0 × P	Zarrow *et al.* (1957)
	R	Decid.	—	2 × P	Zarrow *et al.* (1958)
	Chick	Oviduct	—	2.5 × P	Zarrow *et al.* (1958)
Pregna-4,11-diene-3,20-dione, 17α-hydroxy-, acetate	Rbt	Cl.	O	1 × AP	Dusza *et al.* (1963)
Pregna-4,11-diene-3,20-dione, 17α-methyl-	—	—	—	Active (> P)	Engel *et al.* (1956)
Pregna-4,16-diene-3,20-dione	Rbt	Cl.	—	Inactive	Butenandt and Schmidt-Thomé (1939), Hegner and Reichstein (1943)
	Rbt	C-A	—	Inactive	Shoppee and Reichstein (1941)
	Rbt	McO.	IU	Mod. activity (< P)	Salhanick and Swanson (1961)
Pregna-4,17(20)-dien-3-one	Rbt	Cl.	SC	0.05 × P	Kincl and Folch-Pi (1962b)
Pregna-4,20(21)-dien-3-one	Rbt	Cl.	SC	< 0.025 × P	Kincl and Folch-Pi (1962b)
Pregn-5-ene-3,20-dione	Rbt	Cl.	—	Inactive	Westphal and Schmidt-Thomé (1936)
Pregn-5-en-20-one	Rbt	Carb. An.	—	Inactive	Lutwak-Mann and Adams (1957)
Pregn-5-en-20-one, 3β-bromo-17α-hydroxy-, acetate	Rbt	Cl.	O	0.2 × ENT	Kincl and Folch-Pi (1962a)
Pregn-5-en-20-one, 3β-chloro-17α-hydroxy-, acetate	Rbt	Cl.	O	0.2 × ENT	Kincl and Folch-Pi (1962a)
Pregn-5-en-20-one, 3β-chloro-6-methyl-16α,17α-isopropylidenedioxy-	Rbt	Cl.	O	Active	Rapala and Kraay (1962)
Pregn-5-en-20-one, 3β-fluoro-	Rbt	Cl.	SC	< 0.05 × P	Kincl and Folch-Pi (1962b)
Pregn-5-en-20-one, 3β-fluoro-17α-hydroxy-, acetate	Rbt	Cl.	O	0.5 × ENT	Kincl and Folch-Pi (1962a)

Pregn-5-en-20-one, 3β-hydroxy-	Rbt	Carb. An.	SC	Inactive	Adams and Lutwak-Mann (1955)
	M	H-F	SU	Active	Nakao *et al.* (1958)
Pregn-5-en-20-one, 3β-hydroxy-6-methyl-16α,17α-isopropylidenedioxy-	Rbt	Cl.	O	0.25 × ENT	Rapala and Kraay (1962)
Pregn-5-en-20-one, 3β-hydroxy-16α,17α-isopropylidenedioxy-	Rbt	Cl.	O	0.17 × ENT	Rapala and Kraay (1962)
Pregn-5-en-20-one, 3β,17α-dihydroxy-, 17-acetate	Rbt	Cl.	O	0.5 × ENT	Kincl and Folch-Pi (1962a)
Pregn-5-en-20-one, 16α,17α-dihydroxy-	Rbt	Cl.	O	Inactive	Rapala and Kraay (1962)
Pregna-5,7-dien-20-one, 3β-hydroxy-	Rbt	Cl.	SC	Inactive	Nes *et al.* (1958)
Pregna-5,7,9(11)-trien-20-one, 3β-hydroxy-	Rbt	Cl.	SC	Inactive	Nes *et al.* (1958)
Anthrapregna-5,7,9,14-tetraen-20-one	Rbt	Cl.	SC	Inactive	Nes *et al.* (1958)

Chapter 3

Anti-Gonadotropic Steroids

RALPH I. DORFMAN AND FRED A. KINCL

I. Introduction

This chapter deals with the activity of steroids which inhibit the biosynthesis and/or release of gonadotropins by the anterior pituitary as measured in parabiotic rats, in rabbits, and in the intact rat. This chapter will deal with the activities of various steroids in certain fertility tests in the rodent. It is recognized that factors other than the decrease of gonadotropin hormone output by the pituitary gland may be involved.

In measuring inhibition of gonadotropin pituitary function, it is of importance to distinguish between the inhibition of the follicle stimulating hormone (FSH) and of the luteinizing hormone (LH). It has been assumed that, in the parabiotic rat, suppression of pituitary hypersecretion by steroids is chiefly a measure of FSH inhibition. From the studies on the functional state of the ovaries of the intact parabionts, it is apparent that both FSH and LH may be decreased. Shipley (1962) noted that the number of corpora lutea (CL) in the intact female

partner decreased if either estrogens or androgens were injected to the other member of the parabiotic pair. In some instances, no corpora lutea, but only follicles were found. These findings appear to indicate that by the parabiotic technique using rats it is either impossible to measure selectively FSH inhibition, or that the rat secretes only a single gonadotropin, which may exercise a dual function both of the follicle stimulating and of the luteinizing hormone. On the other hand, it has been reported that various synthetic progestational substances inhibit luteinization in the intact adolescent (30- to 35-day-old) female rat, whereas, the same steroids were found to be relatively inactive when assayed in the parabiotic pair (Shipley, 1962). Thus our present assay methods are not sufficiently refined for establishing the mechanism of action. Nevertheless, the parabiotic rat technique appears to be useful as a rapid, relatively simple method for measuring the overall effect. At the same time, depending upon whether the castrated partner is male or female, it is possible to obtain a rough indication of the androgenic, or estrogenic, activity of the test substance by evaluating the responses of the seminal vesicles and ventral prostate or uterus, respectively.

In evaluating available data, the authors found it necessary to make certain selections. In the parabiotic rat assay, considerable variation in sensitivity may exist depending upon whether the compounds are administered for 7 or 10 days (Shipley, 1962). The stated activity of 19-norandrostene derivatives may be open to question since many of these steroids, until recently, had been synthesized from ring A phenolic steroids and hence contaminated with estrogenic substances.

The effect of steroids in inhibiting the synthesis and/or release of gonadotropins may be transitory, or permanent depending upon the age of the animal. In the mature or the adolescent animal, such inhibition is usually not permanent, and upon cessation of the therapy, gonadal function is usually restored to normal within varying periods of time. In the very young animal, short term steroid treatment in the female (Barraclough and Leathem, 1954; Leatham, 1958) and in the male (Kincl *et al.*, 1962) produces an apparently irreversible inhibition of the gonadal function, which was ascribed, at least in the female, to be due to the effect of steroids on the central nervous system, rather than on the pituitary gland (Barraclough and Gorski, 1961)

II. Activity Assayed in the Parabiotic Rat

Quantitative measurement of the amounts of steroids required to inhibit gonadotropin production in the parabiotic rat, has been used by

Meyer and Hertz (1937) for estrogens, Hertz and Meyer (1937) for androgens, and Biddulph *et al.* (1940) for progesterone, estradiol-17β, and estriol. These early reports demonstrated that, using standard techniques, this method is suitable for quantitative measurements and statistical treatment. Gonadal hormones in general were found to be effective in inhibiting gonadotropin release by the pituitary gland, but larger quantities of androgens, rather than estrogens, were apparently needed to effect the inhibition. Progesterone, though effective, was found to be only weakly active as compared to estrogens and androgens.

Throughout this section, androgenic activity has been referred to only insofar as it was deemed proper to demonstrate the separation between gonadotropin inhibition and androgenic response. Unless otherwise stated, the androgenic potency, as used, represents the average of both the seminal vesicles and ventral prostate responses obtained in the castrated member of the parabiotic pair.

A. ANDROSTANE DERIVATIVES

1. *The Activity of Testosterone*

Daily subcutaneous administration to 30-day-old animals of 80 μg of testosterone (17β-hydroxyandrost-4-en-3-one, I) for the period of 7 days, produced complete inhibition of gonadotropins, defined as the dose which inhibited ovarian growth to unstimulated levels. When the duration of the test was increased to 10 days, higher doses of 160–320 μg per day had to be employed. The 7-day assay was stated to give a sharper end point, measured by the suppression of ovarian weights; the functional state of the ovaries, evaluated by gross macroscopic examination revealed that corpora lutea were not present (Shipley, 1962). Daily dosages of 30 μg and 40 μg of testosterone were reported to produce statistically significant decreases in ovarian weights following 10-day subcutaneous administration (Kincl *et al.*, 1961; Dorfman *et al.*, 1962). The latter authors used the responses obtained upon administration of graded doses of testosterone in evaluating the activity of various steroids (vide infra). All these investigators noted that the dose of testosterone which produced a decrease in ovarian weights in the intact female parabiont was sufficient to stimulate the growth of the accessory sex organs in the castrated, male partner.

The esters of testosterone were found to be more potent than the parent steroid. Testosterone propionate (17β-propionoxyandrost-4-en-3-one) was judged to be about 1.5 times as active as the free alcohol (I), but may be only as androgenic as the primary standard (Table II).

2. *Simple Testosterone Derivatives*

The effects of a simple modification of the testosterone molecule on anti-gonadotropic activity is presented in Tables I and II. In Table I reduction of the Δ^4 double bond apparently did not decrease the activity since compound II possesses the same order of activity as did testosterone, since a total of 200 μg was active. Oxidation of the 17β-hydroxy function to a ketone (androst-4-ene-3,17-dione, III) led to a significant decrease in potency. The activity of III was also studied by Dorfman (1959), who reported about 16% the activity of testosterone (Table II),

TABLE I

ANTI-GONADOTROPIC ACTIVITY OF VARIOUS STEROIDS ADMINISTERED SUBCUTANEOUSLY IN A RAT PARABIOSIS ASSAY[a]

Compound	Number of tests	Total minimum dose to produce ovarian inhibition (mg)
17β-Hydroxy-5α-androstan-3-one (II)	3	0.2
Androst-4-ene-3,17-dione (III)	2	0.8
5α-Androstane-3,17-dione	1	3.0
3α-Hydroxy-5α-androstan-17-one (androsterone)	1	3.0

[a] These data are based on studies performed by various laboratories for the Endocrine Evaluation Branch, Cancer Chemotherapy, National Service Center, National Institutes of Health, Bethesda, Maryland.

and by Shipley (1962), from whose data the activity of III may be estimated to be about one-fourth that of testosterone and about the same as that of androsterone (3α-hydroxy-5α-androstan-17-one, IV).

3. *Synthetic Androgens*

a. 3-Oxo compounds. Kincl *et al.* (1961) and Dorfman and Kincl (1963) studied a number of steroids derived from testosterone and 17β-hydroxy-5α-androstan-3-one and reported a separation of anti-gonadotropic and androgenic activities (Table II).

Two closely related C-2 substituted compounds, 2α-hydroxymethyl-17β-hydroxy-5α-androstan-3-one (V) and 2α-methyl-17β-hydroxy-5α-androstan-3-one (VI) were found to be as potent anti-gonadotropic steroids as testosterone, but significantly less androgenic. The separation

TABLE II

RELATIVE ANTI-GONADOTROPIC AND ANDROGENIC POTENCY OF TESTOSTERONE DERIVATIVES ADMINISTERED SUBCUTANEOUSLY IN A RAT PARABIOSIS ASSAY

Compound administered	Relative potency: Anti-gonadotropic activity	Relative potency: Androgenicity	Reference[a]
Testosterone (I)	100	100	—
Testosterone propionate	140	90	1
Androst-4-ene-3,17-dione (III)	16	35	2
2α-Fluorotestosterone	40	25	1
2α-Fluoro-17α-ethynyl-17β-hydroxyandrost-4-en-3-one	30	15	1
2α-Fluoro-17α-hydroxy-5α-androstan-3-one	30	15	1
2α-Hydroxymethyl-17α-hydroxy-5α-androstan-3-one (V)	120	25	3
2α-Methoxymethyl-17β-hydroxy-5α-androstan-3-one	30	10	1
2α-Methyl-17α-hydroxy-5α-androstan-3-one (VI)	95	38	2
17β-Acetoxy-2-methyl-5α-androst-1-en-3-one	280	95	3
2-Methoxymethylene-17α-hydroxy-5α-androstan-3-one	7	5	2
2-Benzoyloxymethylene-17α-methyl-17β-hydroxy-5α-androstan-3-one	60	25	1
2-Methylene-17α-methyltestosterone	70	45	3
2-Cyano-17α-methyl-5α-androst-1-en-3-one	50	$\leqslant 10$	3
3-[6′-Fulvene]-5α-androstan-17β-ol	40	$\leqslant 5$	3
5-Methyl-17β-hydroxyandrostan-3-one	40	$\leqslant 20$	3
16α,17α-Dimethyl-17α-hydroxy-5α-androstan-3-one	200	25	3
6α-Fluorotestosterone (VII)	350	185	3
6α-Chlorotestosterone acetate (VIII)	375	155	3

[a] Key to References:
1. Kincl *et al.* (1961). 2. Dorfman (1959). 3. Dorfman and Kincl (1963).

in activity was judged to be tenfold and threefold, respectively. Substitution by a halogen atom, by a methylene group, and esterification of the hydroxymethyl group in the C-2 position led to a decrease in activity (Table II); however, an introduction of a halogen or methyl group at C-6 produced active substances; 6α-fluoro-17β-hydroxyandrost-4-en-3-

one (VII) and 6α-chloro-17β-acetoxyandrost-4-en-3-one (VIII) were found to be four times as active as testosterone, and 6α,17α-dimethyl-17β-hydroxy-5α-androstan-3-one (IX) 3.3 times as active. The 17-methyl-ether of compound VI exhibited only weak activity, 20% that of testosterone (Kincl *et al.*, 1961).

The activity of 2α,17α-dimethyltestosterone and of *D*-homotestosterone propionate was studied by Shipley (1962). From the published data, the anti-gonadotropic activity of these two steroids may be estimated at 20% and 60% of testosterone, respectively; the androgenic activity of the two compounds was found to be approximately of the same order, and no separation between the two end points was noted. Additional data pertaining to the anti-gonadotropic activity of various steroids in a parabiosis assay are given in Table III.

TABLE III

ANTI-GONADOTROPIC ACTIVITY OF VARIOUS STEROIDS ADMINISTERED SUBCUTANEOUSLY IN A PARABIOSIS ASSAY[a]

Compound	Number of tests	Minimum total dose to produce ovarian inhibition (mg)
2α-Methyl-17β-propionoxy-5α-androstan-17β-ol	1	0.12
2α-Methyl-17β-hydroxy-5α-androstan-17β-ol	2	0.3
6α-Fluorotestosterone acetate	2	0.4
2α,17α-Dimethyl-17β-hydroxy-5α-androstan-3-one	1	0.6
2α-Methyl-17β-hydroxy-5α-estran-3-one	1	0.6
2-Methyl-17β-hydroxy-5α-androst-1-en-3-one	1	0.6
6β-Methyl-17β-propionoxy-5α-androstan-3-one	1	0.6
6α-Methyltestosterone	1	0.6
6β-Methyltestosterone	1	0.6
4-Chloro-17α-methyl-testosterone	1	0.6
5α-Androst-2-en-17β-ol	1	2.0
2α-Methyl-5α-androstane-3,17-dione	2	3.0
3α-Hydroxy-5α-androstan-17-one	1	3.0
2α,17α-Dimethyl-17β-hydroxyandrost-4-en-3-one	1	6.0
2α-Methoxymethyl-17β-hydroxy-5α-androstan-17β-ol	2	20.0
2-Hydroxymethyl-17β-hydroxy-5α-androstan-3-one	1	30.0
Progesterone	1	30.0

[a] These data are based on studies performed by various laboratories for the Endocrine Evaluation Branch, Cancer Chemotherapy, National Service Center, National Institutes of Health, Bethesda, Maryland.

A number of synthetic 3-oxo androgens was reported to be weakly active anti-gonadotropins. These include 4-chloro testosterone acetate (Clini *et al.*, 1958) and a variety of steroids possessing various molecular modifications. This is illustrated in Table IV, which lists steroids judged to be less than 10% as active as testosterone. Several inactive compounds which were evaluated in a 10-day parabiosis assay follow (the values in

TABLE IV

STEROIDS POSSESSING LESS THAN 10% THE ANTI-GONADOTROPIC ACTIVITY OF TESTOSTERONE WHEN ADMINISTERED SUBCUTANEOUSLY IN A RAT PARABIOSIS ASSAY[a]

Substitution	Steroid
C-1	1-Cyanoandrost-4-ene-3,17-dione
	1-Thioacetylandrost-4-ene-3,17-dione
C-2	2-Hydroxymethylen-17α-ethynyltestosterone
	2-Hydroxymethylene-17α-propinyltestosterone
	2,2,17α-Trimethyl-17β-hydroxy-5α-androstan-3-one
	2-*N*-Methylanilinomethylene-17α-methyl-17β-hydroxy-5α-androstan-3-one
	2α-Dimethylaminptestosterone acetate
	2α-Dimethylaminotestosterone acetate *N*-methyliodide
C-3	Testosterone propionate-3-(*p*-toluene)-thio enolether
C-5	5α-Cyano-17β-hydroxyandrostan-3-one
	5α-Carbamido-17β-hydroxyandrostan-3-one
C-6	6β-Nitrotestosterone acetate
	6α-Nitrotestosterone
	6-(*p*-toluene)thiotestosterone propionate

[a] Data from Kincl *et al.* (1961).

parentheses indicate the total dose in milligrams at which each compound was studied); 6β,17β-diacetoxyandrost-4-en-3-one (100); 17α-oxo-5α-androstane-3,17-dione (30); *D*-homo-3β-hydroxy-17α-oxo-5α-androstan-17-one (30); *D*-homo-17α-oxo-5α-androstan-3β-ol (40).

b. 3-Deoxy steroids. Table V lists nineteen 3-deoxy steroids which were studied by Kincl and Dorfman (1964). Ten compounds were found to be either more active or as active as testosterone as anti-gonadotropins in a parabiosis assay. All these compounds were either

TABLE V

RELATIVE POTENCY OF VARIOUS 3-DEOXY STEROIDS ADMINISTERED SUBCUTANEOUSLY IN A RAT PARABIOSIS ASSAY[a]

Steroid	Relative potency: Anti-gonadotropic activity	Androgenic
Testosterone	100	100
2-Cyano-5α-androst-2-en-17β-ol acetate (X)	400	65
2-Cyano-5α-androst-2-en-17β-ol	350	80
2-Cyano-17α-methyl-5α-androst-2-en-17β-ol	350	110
2-Formyl-5α-androst-2-en-17β-ol (XI)	250	10
2-Hydroxymethyl-5α-androst-2-en-17β-ol	220	20
2-Methyl-5α-androst-2-en-17β-ol	150	25
17α-Methyl-5α-androst-2-en-17β-ol	150	50
2,17α-Dimethyl-5α-androst-2-en-17β-ol	130	≤ 15
2-Formyl-17α-methyl-5α-androst-2-en-17β-ol	100	≤ 10
Androsta-2,4-dien-17β-ol acetate	100	45
2α-Formyl-5α-androstan-17β-ol (XII)	60	< 10
2-Acetoxymethylen-5α-androst-3-en-17β-ol acetate	50	20
2-Methylen-5α-androstan-17β-ol acetate	35	10
17α-Methylandrosta-3,5-dien-17β-ol	20	5
Androst-5-en-17β-ol	20	6
2-Hydroxyethyl-5α-androst-2-en-17β-ol	20	< 10
2α,17α-Dimethylandrosta-3,5-dien-17β-ol	20	20
Androst-4-en-17β-ol acetate	15	10
2-Chloromethyl-5α-androst-2-en-17β-ol acetate	15	< 10

[a] Data Kíncl and Dorfman (1964).

unsaturated at carbon 2, or had a substituent at this position. The most active compound in this series was 2-cyano-5α-androst-2-en-17β-ol acetate (X) which was four times as potent as testosterone. Twenty-five-fold separation between gonadotropin inhibition and androgenic potency was observed for 2-formyl-5α-androst-2-en-17β-ol (XI). Saturation of the double bond decreased the potency apparently since 2α-formyl-5α-androstan-17β-ol (XII) was only one-fourth as active as the corresponding Δ^2-derivative (XI).

The influence of various substitutions on the anti-gonadotropic-androgenic ratio is presented in Table VI. The 2-formyl derivative had the remarkably high ratio of 25 on a scale where testosterone was assigned the value of 1.

TABLE VI

THE INFLUENCE OF VARIOUS SUBSTITUENTS ON THE ANTI-GONADOTROPIC-ANDROGENIC RATIO OF 17β-HYDROXY-5α-ANDROST-2-ENE DERIVATIVES

Substituent	Anti-gonadotropic-androgenic ratio (testosterone = 1)
2-Formyl	25.0
2-Hydroxymethyl	11.0
2-Formyl-17α-methyl	≥ 10.0
2,17α-Dimethyl	≥ 8.7
2-Methyl	6.0
2-Cyano	4.4
17α-Methyl	3.0
2-Cyano-17α-methyl	3.2

c. Oral activity. The parabiotic rat technique may be used to evaluate orally administered compounds. In the 10-day test, significant gonadotropin inhibition is achieved with an oral daily dose of 250 μg of methyltestosterone (Dorfman and Kincl, 1963). Using this steroid as a reference standard, Dorfman and Kincl (1963) and Kincl and Folch-Pi (1962) evaluated the oral activity of nine steroids (Table VII).

Two compounds, 2-hydroxymethyl-17α-methyl-5α-17β-hydroxyandrostan-3-one (XIII) and 17α-methyl-17β-hydroxyandrosta-1,4-dien-3-one (XIV) were only weakly active, i.e., about one-fifth the activity of methyltestosterone, and exhibited no separation in anti-gonadotropic-androgenic activity. 17α-Ethylestr-4-en-17β-ol (XV) was found to exhibit 70% the inhibitory activity of methyltestosterone at a dose which did not stimulate the growth of the accessory sex glands. 17α-Methylandrost-4-en-3β,17β-diol possessed 45% the gonadotropin inhibitory, and 85% the androgenic, activity of methyltestosterone.

B. ESTRANE DERIVATIVES

1. *The Activity of Ring A Phenolic Steroids*

Estrogens are particularly potent anti-gonadotropins. Whereas the minimal dose of testosterone needed to produce an inhibition of the pituitary gonadotropin function in the castrated parabiont is 80 μg per day, under similar conditions, 1/160th of this dose, i.e., 0.5 μg of estrone per day, produced comparable anti-gonadotropic effects (Shipley, 1962). Table VIII lists the minimal amounts of estradiol-17β and estriol

needed to produce gonadotropin inhibition (Biddulph *et al.*, 1940). Dorfman and Dorfman (1963) found 0.5 μg of estrone as the minimum

TABLE VII

RELATIVE POTENCY OF VARIOUS STEROIDS ADMINISTERED ORALLY IN A RAT PARABIOSIS ASSAY

Compound administered	Relative potency: Anti-gonadotropic activity	Relative potency: Androgenic	Reference[a]
Methyltestosterone	100	100	—
2-Methylene-17α-methylandrostan-17β-ol	200	65	2
17α-Ethylestr-4-en-17β-ol	75	—	1
17α-Methylandrost-2-en-17α-ol	60	55	2
17α-Methyl-androst-4-en-3β,17β-diol	45	85	1
2-Hydroxymethyl-17α-methylandrost-2-en-17β-ol	25	35	2
17α-Methyl-17β-hydroxyandrosta-1,4-dien-3-one	20	20	1
2-Hydroxymethylene-17α-methyl-17β-hydroxy-5α-androstan-3-one	20	20	1
2-Formyl-17α-methylandrost-2-en-17β-ol	15	35	2
2-Cyanoandrost-2-en-17β-ol acetate	< 10	25	2

[a] Key to References
1. Kincl and Folch-Pi (1962). 2. Dorfman and Kincl (1963).

TABLE VIII

MINIMAL DOSE OF VARIOUS ESTROGENS REQUIRED TO INHIBIT SECRETION OF GONADOTROPINS IN PARABIOTIC RATS[a]

Compound administered	Parabionts	Daily dose (μg)
Estradiol-17β	ovariectomized–intact female	0.0175
	castrated male–intact female	0.15
Estriol	ovariectomized–intact female	0.3
	castrated male–intact female	8.0

[a] Data from Biddulph *et al.* (1940).

total dose to inhibit pituitary gonadotropin secretion in the ovariectomized female rat in parabiosis with an intact female.

The authors noted that the castrate female–intact female pair was more sensitive to estrogen treatment than was the castrate male–intact female combination. Other groups investigated the activity of estrogens and reported the effective dose for estrone to be 0.05–0.2 μg/day (Meyer and Hertz, 1937), 0.075 μg/day (Byrnes and Meyer, 1951), and 0.065 μg/day for estradiol (Byrnes *et al.*, 1951).

The activity of several 3-deoxy estrogens in the spayed female–intact female parabiotic pair was studied by Dorfman *et al.* (1962). 4,17α-Estra-1,3,5(10)-triene-17β-ol was found to be 1/500th as active as estrone and possessed approximately the same degree of estrogenic potency; hence, the pituitary suppressing activity of this compound was related to its estrogenicity.

17α-Ethynylestradiol-17β 3-methylether (3-methoxy-17α-ethynylestra-1,3,5(10)-trien-17β-ol, XVI) was fully active as 1 μg/day by subcutaneous injection in the male–female parabiotic pair (Dorfman and Kincl, 1963).

Faleoni and Ercoli (1963) found the following compounds to produce anti-gonadotropic effects in a 10-day test using the oral administration route: 17α-ethynylestradiol-17β, 1.48 μg (total dose); 17α-ethynylestradiol-17β-methylether, > 1.55; and 17α-ethynylestradiol-17β, 3-cyclopentylether, 1.85 μg.

2. *Derivatives of 19-Nortestosterone*

In considering the activity of 19-nortestosterone derivatives, determined in the parabiotic rat, it should be noted that until recently ring A phenolic steroids had to be used as starting material in the syntheses. The available analytical methods did not usually permit detection of the small, but highly active, estrogen impurities, hence data obtained on these materials may be open to revision since estrogens are highly active anti-gonadotropins.

Epstein *et al.* (1958) reported on the activity of 17α-alkyl-19-nortestosterone derivatives, using the minimal effective dose needed to produce significant suppression of the ovarian weights as a means of evaluating potency. 17α-Ethyl-17β-hydroxyestr-4-en-3-one (XVII) was reported as the most active compound; 10 μg/day proved to be the effective dose. Ten times as much (100 μg/day) was required of 17α-methyl-19-nortestosterone (17α-methyl-17β-hydroxyestr-4-en-3-one, XVIII) and of 17α-ethynyl-17β-hydroxyestr-5(10)-en-3-one (XIX). Norethisterone, 17α-ethynyl-17β-hydroxyestr-4-en-3-one (XX), was stated to be ineffective at a sixfold dose of the $\Delta^{5(10)}$ analog (XIX). The

activity also of compound XVIII was studied by Shipley (1962), who reported the effective subcutaneous dose as 114 to 172 μg/day in the 7-day test, i.e., about 30% the activity of testosterone. Under similar conditions, 19-nortestosterone cyclopentylpropionate was found to be approximately as active as testosterone. Another 19-nortesterone ester (19-nortestosterone benzoate) was judged by Lerner *et al.* (1959) to be only one-tenth as active as testosterone propionate.

Additional substitutions on the 17α-ethynyl group increased (Fried *et al.*, 1961) the potency as compared to the parent steroid (Table IX).

TABLE IX

ANTI-GONADOTROPIC ACTIVITY OF 17α-SUBSTITUTED 19-NORTESTOSTERONE DERIVATIVES ADMINISTERED ORALLY[a]

	Relative potency (norethisterone = 1)		
17α-Substitution	Δ^4	$\Delta^{5(10)}$	$\Delta^{4,9}$
—C≡CH	1	2–3	1.5
—C≡CCF_3	2–3	5–6	—
—C≡CCl	3	3–4	6–8
—C≡CBr	1–2	—	—
—CF=CF_2	< 1	—	—

[a] Data from Fried *et al.* (1961).

The authors reported higher oral activity for $\Delta^{5(10)}$ compounds than for the Δ^4 steroids and found that it was further increased if two centers of unsaturation were present. For all compounds, the activity was apparently independent of the oral progestational activity as measured in the McPhail test, except for one steroid, 17α-chloroethynyl-17β-hydroxyestra-4,9-diene, which was stated to be a potent oral progestogen. A closely related steroid, 17α-chloroethyl-17β-hydroxyestra-4,9-dien-3-one was reported (Steelman *et al.*, 1962) to possess eight times the oral anti-gonadotropic activity of norethisterone; the oral progestational activity was stated to be increased by approximately the same order.

17α-Chloroethynyl-17β-hydroxy-4,9-dien-3-one was judged to be six to eight times more potent than norethisterone, while the corresponding -4,9,8(14)-triene was 1.5–2 times as active as the same standard (Windholz *et al.*, 1963).

Activity of other 19-nortestosterone derivatives is given in Table X (Kincl *et al.*, 1961); the potency of several 3-deoxyestran derivatives is described in Table XI (Kincl and Dorfman, 1964).

TABLE X

RELATIVE ANTI-GONADOTROPIC ACTIVITY OF 19-NORTESTOSTERONE DERIVATIVES ADMINISTERED SUBCUTANEOUSLY[a] IN A RAT PARABIOSIS ASSAY

Compound administered	Relative potency (testosterone = 100): Anti-gonadotropic activity	Androgenicity
4-Chloro-17α-methyl-17β-hydroxyestr-4-en-3-one	100	16
2α-Methyl-17β-hydroxy-5α-estran-3-one	70	20
16β-Methyl-17β-hydroxyester-4-en-3-one	30	10
2,2,17α-Trimethyl-17β-hydroxy-5α-estran-3-one	5	5
2,2,17α-Trimethyl-5α-estrane-3β,17β-diol	< 10	< 10

[a] Data from Kincl *et al.* (1961).

TABLE XI

RELATIVE ANTI-GONADOTROPIC ACTIVITY OF 3-DEOXY ESTRONE DERIVATIVES ADMINISTERED SUBCUTANEOUSLY[a]

Compound administered	Relative potency (testosterone = 100): Ovarian weight	Androgenic
2-Cyanoestr-2-en-17β-ol acetate	300	40
2-Hydroxymethylestr-2-en-17β-ol	160	≦ 15
2-Formylestr-2-en-17β-ol	40	< 10

[a] Data from Kincl and Dorfman (1964).

17α-Ethylestr-4-en-17β-ol (XV) was reported to be a potent pituitary inhibitor when administered orally to the parabiotic rat (Kincl and Folch-Pi, 1962; vide supra). 5ξ-Methyl-17β-hydroxyestran-3-one was found to be about one-fifth as anti-gonadotropic as testosterone and less than one-twentieth as androgenic (Dorfman and Kincl, 1963).

C. Pregnane Derivatives

It has already been mentioned that although pregnane derivatives are capable of inhibiting the pituitary gonadotropin function in the parabiotic rat, the doses needed to achieve this effect are generally considerably greater than those of estrogens or androgens. Biddulph *et al.* (1940) and Byrnes *et al.* (1951) reported a significant decrease in ovarian weights in parabionts following the daily subcutaneous administration of 1 mg of progesterone (pregn-4-ene-3,20-dione, XXI) and Shipley (1962) obtained gonadotropin inhibition following the subcutaneous administration of 30 mg of this steroid in seven equally divided doses. Approximately the same degree of inhibition was reported by Shipley for the 21-fluoro derivative (21-fluoropregn-4-ene 3,20-dione); pregna-4,16-diene-3,20-dione (XXII) was found to be less active. In contrast, Epstein *et al.* (1958) found no inhibition in the 10-day test with progesterone given at 40 mg/day or with 17α-hydroxy-

TABLE XII

Gonadotropin Inhibiting Activity of Various Pregnane Derivatives Administered Subcutaneously in a Rat Parabiosis Assay

Compound	Minimum total dose required (mg)	Reference[a]
Progesterone (XXI)	30.0	1
4,9α-Dichloropregn-4-ene-3,11,20-trione	40.0	1
6α-Chloro-17α-acetoxypregn-1,4-diene-3,20-dione	30.0	1
19-Norprogesterone	20.0	1
Deoxycorticosterone	4 25	3
3β-Acetoxy-16β-carboxamide-(17α)-isopregn-5-en-20-one	1.0	1
16α,17α-Oxidopregn-4-ene-3,17-dione	> 30.0	2
Pregna-4,16-diene-3,20-dione	> 30.0	2
4-Chloro-17α-hydroxypregn-4-ene-3,20-dione	> 21 0	2
Cortisone	> 20.0	3
6-Chloro-17α-acetoxypregna-4,6-diene-3,20-dione	> 10.0	1
6-Chloro-16α-methyl-17α-acetoxypregna-4,6-diene-3,20-dione	> 10.0	1

[a] Key to References:
1. Kincl and Dorfman (1963).
2. CCNSC data (Cancer Chemotherapy National Service Center).
3. Byrnes and Shipley (1950).

progesterone caproate at 10 mg/day administered subcutaneously. The activities of other derivatives are listed in Table XII.

Falconi *et al.* (1961) reported progesterone 3-cyclopentylenolether as inactive at an oral dose of 2.2 mg per day. In a similar test, 6α-methyl-17α-acetoxyprogesterone (XXII) was found active at 1.93 mg per day dose level.

D. Gonadotropin Inhibition Related to Androgen-Myotrophic Activity

Table XIII summarizes the relationship between anti-gonadotropic, androgenic, and myotrophic activity (judged on basis of levator ani

TABLE XIII

Relationship between Anti-Gonadotropic, Androgenic, and Myotrophic Activity in a Rat Parabiosis Assay (Subcutaneous Administration)

Compound administered	Anti-gonadotropic activity (AGA)	Ratios AGA/MA	AGA/AA[a]	Myotrophic (levator ani) activity (MA)
Testosterone	100	1.0	1.0	100
2-Cyano-5α-androst-2-en-17β-ol acetate	400	1.33	6.15	300
6α-Chlorotestosterone	375	1 38	2.4	270
6α-Fluorotestosterone	350	1.8	1.9	190
2-Cyano-5α-androst-2-en-17β-ol	350	1.4	4.4	250
2-Cyano-17α-methyl-5α-androst-2-en-17β-ol	350	1.0	3.2	350
2-Cyano-5α-estr-2-en-17β-ol acetate	300	3.0	7.5	100
17β-Acetoxy-2-methyl-(5α)-androst-1-en-3-one	280	7.7	2.9	36
2-Formyl-5α-androst-2-en-17β-ol	250	1.66	25.0	150
2-Hydroxymethyl-5α-androst-2-en-17β-ol	220	1.0	11.0	220
6α,17α-Dimethyl-17β-hydroxy-5α-androstan-3-one	200	4.0	7.4	50
2-Hydroxymethyl-5α-estr-2-en-17β-ol	160	3.2	>11.0	50
2-Methyl-5α-androst-2-en-17β-ol	150	1.5	6.0	100

[a] AA, androgenic activity.

continued

TABLE XIII—*continued*

Compound administered	Anti-gonadotropic activity (AGA)	Ratios AGA/MA	Ratios AGA/AA[a]	Myotrophic (levator ani) activity (MA)
17α-Methyl-5α-androst-2-en-17β-ol	150	1.0	3.0	150
17β-Propionoxyandrost-4-en-3-one	140	1.4	1.5	100
2,17α-Dimethyl-5α-androst-2-en-17β-ol	130	1.3	≧8 6	100
2α-Hydroxymethyl-17β-hydroxy-5α-androstan-3-one	120	1.2	4.0	100
4-Chloro-17α-methyl-17β-hydroxy-5α-estran-3-one	100	1.4	6.2	70
2-Formyl-17α-methyl-5α-androst-2-en-17β-ol	100	1.0	≧10.0	100
Androsta-2,4-dien-17β-ol acetate	100	1.0	2.2	100
2-Methylene-17α-methyl-17β-hydroxyandrost-4-en-3-one	70	0 7	≧1.5	100
2α-Methyl-17β-hydroxy-5α-estran-3-one	70	1.7	3.5	40
2α-Formyl-5α-androstan-17β-ol	60	1.5	≧6.0	40
2-Benzethoxymethylene-17α-methyl-5α-androstan-17β-ol	60	3.0	2 4	20
2-Acetoxymethylen-5α-androst-3-en-17β-ol acetate	50	2.5	2.5	20
2-Cyano-17α-methyl-17β-hydroxy-5α-androst-1-en-3-one	50	1 6	≧5 0	30
5ξ-Methyl-17β-hydroxy-androstan-3-one	40	2.0	≧2.3	20
3-[6′-Fulvene]-(5α)-androstan-17β-ol	40	6.6	≧6.6	6
2-Formyl-5α-estr-2-en-17β-ol	40	1.3	≧4.0	30
2α-Fluoro-17β-hydroxyandrost-4-en-3-one	40	1.3	1.6	30
2-Methylene-5α-androstan-17β-ol acetate	35	1.7	3.5	20
16β-Methyl-17β-hydroxyestr-4-en-3-one	30	0.75	3.0	40
2α-Methoxymethyl-17β-hydroxy-5α-androstan-3-one	30	1.0	3.0	30
2α-Fluoro-17β-hydroxy-5α-androstan-3-one	30	1.5	2.0	20

[a] AA androgenic activity.

TABLE XIII—*continued*

Compound administered	Anti-gonadotropic activity (AGA)	Ratios AGA/MA	Ratios AGA/AA[a]	Myotrophic (levator ani) activity (MA)
2α-Fluoro-17α-ethynyl-17β-hydroxyandrost-4-en-3-one	30	3.0	2.0	10
17α-Methylandrosta-3,5-dien-17β-ol	20	1.0	4.0	20
Androst-5-en-17β-ol	20	2.0	3.3	<10
2-Hydroxyethyl-5α-androst-2-en-17β-ol	20	2.0	≧2.0	<10
2α,17α-Dimethylandrosta-3,5-dien-17β-ol	20	0.4	1.0	50
5-Methyl-17β-hydroxyestran-3-one	20	1.4	≧4.0	14
2α-Methyl-5α-androstan-17β-ol-17-methylether	20	1.0	2.0	20
6α-Chloro-17α-acetoxypregna-diene-3,20-dione	20	10.0	40.0	2
Androst-4-en-17β-ol- acetate	15	1.5	≧1.5	10
2-Chloromethyl-5α-androst-2-en-17β-ol acetate	15	1.5	≧1.5	10
2-Cyano-5α-androst-2-en-17β-ol acetate	9	≧1.0	≧1.0	—
4,9-Dichloropregn-4-ene-3,11,20-trione	8	1.1	1.3	8
2-Methoxymethylene-17β-hydroxy-5α-androst-3-one	7	1.7	1.4	4
2,2,17α-Trimethyl-17β-hydroxy-5α-estran-3-one	5	2.5	1.2	2
2,2,17α-Trimethyl-17β-hydroxy-5α-androst-3-one	5	2.5	1.2	2

[a] AA, androgenic activity.

weight) of compounds administered subcutaneously, and Table XIV lists compounds studied by gavage (Kincl *et al.*, 1962; Dorfman and Kincl, 1963). These tables also list the respective ratios of anti-gonadotropic activity to myotrophic, and anti-gonadotropic to androgenic. A strong positive correlation exists between the relative potency of the steroids on the parameters anti-gonadotropic and myotrophic (rat levator ani) activity. This is evident when one considers the mean anti-gonadotropic–myotrophic ratio among 47 compounds of Table XIII, which is 1.87. The corresponding ratio of 4.67 for the anti-gonadotropic ratio indicates a relatively lower androgenic activity than

TABLE XIV

RELATIONSHIP BETWEEN ANTI-GONADOTROPIC, ANDROGENIC, AND MYOTROPHIC ACTIVITY IN A PARABIOSIS ASSAY
(ORAL ADMINISTRATION)

Compound administered	Anti-gonadotropic activity	Ratios AGA/MA	Ratios AGA/AA[a]	Myotrophic (levator ani) activity (MA)
2-Methylene-17α-methyl-androstan-17β-ol	200	0.43	3.0	460
17α-Methylandrost-2-en-17β-ol	60	2.4	1.1	25
2-Hydroxymethyl-17α-methyl-androst-2-en-17β-ol	25	0.12	0.71	200
2-Formyl-17α-methylandrost-2-en-17β-ol	15	1.5	0.42	10
2-Cyanoandrost-2-en-17β-ol acetate	< 10	> 0.66	> 0.40	15
6α-Methyl-17α-acetoxypregna-1,4-diene-3,20-dione	8	0.8	0.8	10
6α-Chloro-17α-acetoxypregna-1,4-diene-3,20-dione	7	0.7	0.7	10
6α-Chloro-17α-acetoxypregna-4-ene-3,20-dione	6	0.6	0.6	10
6-Chloro-17α-acetoxypregna-4,6-diene-3,20-dione	4	0.4	0.4	10

[a] AA, androgenic activity.

myotrophic activity among these steroids. Analysis of the correlation coefficient among the 47 compounds yields a value of λ, indicating a strong association between these parameters.

Table XIVA summarizes the relative anti-gonadotropic potencies of various steroids in a rat parabiosis assay by subcutaneous injection (Shipley, 1962). All compounds were less active than the reference standard testosterone.

III. Activity Assayed in the Parabiotic Mouse

Miyake *et al.* (1961) used the parabiotic mouse to evaluate the activity of various steroids. The test compounds, suspended in aqueous medium, were studied both by subcutaneous injection and gavage. Decrease in the ovarian weights was used as the measure of pituitary gonadotropin suppression. From the data of these workers it is not

TABLE XIVA

THE RELATIVE ANTI-GONADOTROPIC ACTIVITY OF VARIOUS STEROIDS IN A RAT PARABIOSIS ASSAY BY SUBCUTANEOUS INJECTION[a]

Steroid	Number of pairs of rats	Relative potency (testosterone = 100)
17α-Ethynyl-19-nortestosterone	10	100
19-Nortestosterone cyclopentyl propionate	10	100
17α-Methyl-19-nortestosterone	20	35
Androsterone	10	20
Androst-4-ene-3,17-dione	10	25
D-Homotestosterone propionate	20	60
2α,17α-Dimethyltestosterone	10	17
Progesterone	10	1.3
Pregna-4,16-diene-3,20-dione	10	0.3
21-Fluoroprogesterone	10	2
11β-Hydroxytestosterone propionate	10	33

[a] Calculated from data of Shipley (1962).

TABLE XV

ANTI-GONADOTROPIC ACTIVITY OF VARIOUS SUBCUTANEOUSLY ADMINISTERED STEROIDS IN A MOUSE PARABIOSIS ASSAY[a]

Steroid	Relative potency
Estrone	1000
17α-Ethynylestradiol-17β	100
17α-Ethynylestradiol-17β 3-methylether (XVI)	100
17α-Ethynyl-17β-hydroxyestr-5(10)-en-3-one (XIX)	100
Diethylstilbestrol	100
Estriol	1
6α-Methyl-17α-acetoxypregn-4-ene-3,20-dione (XXII)	1-10
Methyltestosterone	1
17α-Methyl-17β-hydroxyandrost-5-ene-3β,17β-diol	1
17α-Ethynyltestosterone	1
17α-Ethyl-19-nortestosterone (XVII)	0.1
17α-Ethynyl-19-nortestosterone (XX)	0.1
2α-Methyl-17β-hydroxy-5α-androstan-17β-ol (VI)	0.01
2-Hydroxymethylene-17α-methyl-17β-hydroxy-5α-androstan-17β-ol (XIII)	0.01
2α,17α-Dimethyl-17β-hydroxy-5α-androstan-17β-ol	0.01
17α-Acetoxypregn-4-ene-3,20-dione	0.01
17α-1-Methallyl-19-nortestosterone	0.01
17α-2-Methallyl-19-nortestosterone	0.01
Progesterone (XXI)	0.001
Cortisone acetate	< 0.01
Cortisol Acetate	< 0.01
Deoxycorticosterone acetate	< 0.001

[a] Data from Miyake *et al.* (1961).

possible to determine the relative potency of the various steroids studied since the selected dose interval was tenfold, but certain relationships between structure and relative potency are indicated. The data are presented in Table XV (subcutaneous injection) and Table XVI (oral administration). These data are similar to those reported for the parabiotic rat: estrogens are more potent than androgens, and pregnane derivatives in general are the least active. Since the authors evaluated

TABLE XVI

ANTI-GONADOTROPIC ACTIVITY OF VARIOUS ORALLY ADMINISTERED STEROIDS ASSAYED IN A MOUSE-PARABIOSIS ASSAY[a]

Steroid	Relative potency
17α-Ethynylestradiol-17β 3-methylether	10,000
Estrone	100
17α-Ethynyl-17β-hydroxyestr-5(10)-en-3-one (XIX)	100
17α-Ethyl-19-nortestosterone (XVII)	10
17α-Ethynyl-19-nortestosterone (XX)	10
17α-2-Methallyl-19-nortestosterone	< 10
Testosterone propionate	1
Methyltestosterone	1
17α-Ethynyltestosterone	1
2-Hydroxymethylene-17α-methyl-17β-hydroxy-5α-androstan-3-one (XIII)	1
17α-1-Methallyl-19-nortestosterone	< 1
Progesterone (XXI)	< 1
6α-Methyl-17α-acetoxypregn-4-ene-3,20-dione (XXII)	< 1

[a] Data from Miyake *et al.* (1961).

a number of steroids both by subcutaneous injection and by oral administration, it is possible to make some analysis of the influences of the route of administration on the relative potency (Table XVII).

Estrone was reported to have only one ten-thousandth the activity of the estrogen by the oral route as compared to the injection route (Table XVII). 6α-Methyl-17α-acetoxyprogesterone showed a remarkably high activity by injection, but even at the 10 mg dose level it was essentially inactive orally.

Table XVIII illustrates comparative data and minimal doses needed to produce inhibition of ovarian weights in the parabiotic rat and mouse.

IV. Anti-Ovulation Activity Assayed in the Rabbit

Ovulation in many animals may be inhibited by an active corpus luteum (Parkes, 1929; Makepeace *et al.* 1937; Boyarsky *et al.*, 1947) and

TABLE XVII

RELATIVE POTENCY OF VARIOUS STEROIDS AS DETERMINED IN A MOUSE PARABIOSIS ASSAY BY ORAL AND SUBCUTANEOUS INJECTION[a]

Steroid	Quantity needed to produce significant suppression (mg): Subcutaneous injection (S)	Oral administration (O)	Ratio S/O
Estrone	0.00001	0.1	0.0001
17α-Ethynylestradiol-17β 3-methylether	0.0001	0.001	0.1
17α-Methyltestosterone	0.01	1	0.01
17α-Ethynyltestosterone	0.01	10	0.001
Progesterone	10	> 10	< 1
6α-Methyl-17α-acetoxypregn-4-ene-3,20-dione	0.0001	> 10	< 0.00001
17α-Ethyl-19-nortestosterone	0.1	1	0.1
17α-Ethynyl-19-nortestosterone	0.1	0.01	10
17α-Ethynyl-17β-hydroxyestr-5(10)-en-3-one	0.00001	0.01	0.001

[a] Data from Miyake *et al.* (1961).

TABLE XVIII

COMPARISON OF EFFECTIVE DOSES (ADMINISTERED BY INJECTION) REQUIRED TO PRODUCE GONADOTROPIN INHIBITION IN THE PARABIOTIC RAT AND MOUSE

Steroid	Total dose needed to produce inhibition (μg): Parabiotic rat	Parabiotic mouse[a]
Estrone	1[d]	0.01
17α-Ethynylestradiol-17β 3-methylether	0.6[d]	0.1
Estriol	8[b]	10
Progesterone	30,000[c]	10,000
2α-Methyl-5α-17β-hydroxy-androstan-3-one	1,000[d]	1,000

[a] From Miyake *et al.* (1961).
[b] From Biddulph *et al.* (1940).
[c] From Shipley (1962).
[d] From Dorfman and Kincl (1963).

by treatment with progesterone or other progestational agents (Makepeace *et al.*, 1937; Astwood and Fevold, 1939). Zondek and Sklow (1941) used an electrically stimulated rabbit doe and were able to inhibit ovulation and corpora lutea formation with a single injection of estradiol-17β, 0.5 mg given 8 hours prior to stimulation, and with a daily dose of 2 mg of testosterone given over a period of 5 days.

Pincus and Chang (1953) developed a relatively standardized technique using the mated doe. In their test, the rabbit was mated 18–24 hours after the test substance was administered in a single dose, either by injection or intravaginally. Proof of ovulation was obtained by laparatomy performed 24 hours after mating by the presence or absence of corpora lutea in the ovaries.

These investigators reported that a single injection of progesterone (30 mg) prevented ovulation for 24 days. Oral doses of 2–10 mg of 17α-ethynyltestosterone were effective. Progesterone and 17α-hydroxyprogesterone were reported to be effective anti-ovulatory substances at 1-mg dose level when administered intravaginally. 17α-Methylprogesterone was effective subcutaneously (5 mg) and intravaginally (5 mg) and inactive by gavage. 3β-Hydroxy-5α-pregnan-20-one, 5 mg, was inactive when tested by intravaginal and subcutaneous injection routes.

A. Pregnane Derivatives

The anti-ovulatory activity of a compound is not related necessarily to the progestational activity of a compound or to the anti-gonadotropic activity as measured in parabiotic rats. Many progestational agents which are relatively inactive, or only weakly active when assayed in the parabiotic rat, are potent anti-ovulatory agents when tested in the rabbit. These compounds appear to block directly the release of the luteinizing hormone from the pituitary, as judged from one report (Kincl, 1963). The same degree of anti-ovulatory activity was observed with 19-norprogesterone (19-norpregn-4-ene-3,20-dione, XXIII) and 6-chloro-6-dehydro-17α-acetoxyprogesterone (6-chloro-17α-acetoxypregna-4,6-diene-3,20-dione, XXIV), regardless of whether the doe was stimulated by coitus or with copper acetate. On the other hand, no inhibition was observed when human chorionic gonadotropin was used to induce the ovulation; this finding appears to eliminate, at least with these two compounds, the direct effect on the ovaries

1. *Activity by Subcutaneous Injection*

Barnes *et al.* (1959) used a rabbit ovulation inhibition test based on the work of Makepeace *et al.* (1937) using an ED_{50} end point. Progeste-

rone by injection at a dose of 1 mg produced 100% inhibition of ovulation. 17α-Acetoxyprogesterone was seven times as active as progesterone, and 6α-methyl-17-acetoxyprogesterone was assessed as 20 times as active as the same standard. Using essentially the method of Pincus and Chang (1953), Kincl and Dorfman (1963) assigned the relative potencies to various steroids derived from progesterone and 17α-acetoxyprogesterone. In this study, norethisterone (XX) was used as the standard.

TABLE XIX

RELATIVE POTENCY OF VARIOUS SUBCUTANEOUSLY ADMINISTERED STEROIDS IN AN ANTI-OVULATION ASSAY[a]

Steroid	Number of rabbits	Relative potency
Norethisterone	52	100
19-Norprogesterone	36	3,000
6-Chloro-17α-acetoxypregna-4,6-diene-3,20-dione (XXIV)	20	800
6-Chloro-17α-acetoxypregna-1,4,6-triene-3,20-dione	11	500
6-Chloro-16α-methyl-17α-acetoxypregna-4,6-diene-3,20-dione	9	280
6α-Chloro-17α-acetoxypregna-1,4-diene-3,20-dione	18	200
17α-Acetoxypregna-4,6-diene-3,20-dione	5	200
Progesterone	30	20
17α-Ethynylestradiol-17β 3-methylether	20	100

[a] Data from Kincl and Dorfman (1963).

19-Norprogesterone (XXIII) was the most active anti-ovulatory steroid found and was judged to be about 150 times more active than progesterone, or 30 times more active than norethisterone. Various derivatives of 17α-acetoxyprogesterone substituted by a halogen atom on C-6, and possessing one or two additional double bonds in the molecule, were also found to be potent anti-ovulatory compounds (Table XIX). Under the conditions of the test, progesterone was found to possess about 20% the activity of compound XX.

Other modifications of the progesterone molecule, such as the reduction of the Δ^4 double bond, of the 3-ketone, introduction of a substituent at C-4, change in conformation of the side chain, produced compounds that were found to be less active than norethisterone (Table XX).

TABLE XX

RELATIVE POTENCY OF WEAKLY ACTIVE SUBCUTANEOUSLY INJECTED STEROIDS IN AN ANTI-OVULATION ASSAY[a]

Steroid	Number of rabbits	Relative potency (norethisterone = 100)
16β-Methyl-17α-pregn-5-en-3β-ol	3	7
3β-Hydroxy-5α-pregnan-20-*N*-acethylhydrazine	6	7
16α-Hydroxymethylpregn-5-en-3β,20-diol diacetate	4	2
4-Chloroprogesterone	5	2
3β-Hydroxy-5α-pregnane-20-nicotinylhydrazine	2	1
3α,17α-Dihydroxy-5β-pregnane-3α,17α-diol-20-one	7	1
3β-Chloro-16α-methylpregn-5-en-20-one	5	1
3β-Hydroxy-16β-methylpregn-5-en-20-one	4	1
Enol lactone of 16α-carboxy-3β-acetoxy-17α-pregna-5,20(21)-diene-20-ol	3	1
17α-Hydroxypregna-1,4-diene-3,20-dione	4	0.5
3β-Chloro-17α-acetoxypregn-5-en-20-one	7	0.5
17α-Hydroxy-5α-pregn-1-ene-3,20-dione	4	0.25
6β-Hydroxyprogesterone	2	0.25

[a] Data from Kincl and Dorfman (1963).

TABLE XXI

RELATIVE POTENCIES OF ANTI-OVULATORY STEROIDS WHICH EXHIBIT SHALLOW DOSE RESPONSE CURVES (SUBCUTANEOUS INJECTION)[a]

Steroid	Total number of rabbits	Range of relative potencies (norethisterone = 100)	
3β-Chloropregn-5-en-20-one	6	10	100
16β-Carboxamide-17α-progesterone	6	7	50
3β-Acetoxy-16β-*N*-diethylcarboxamidepregn-5-en-20-one	7	2	30
3β-Hydroxy-21-methylene-*N*-piperidyl-5α-pregnan-20-one	8	1	10
Allopregnan-3β-ol-20-isonicotinylhydrazone	13	2	7
Allopregnan-3β-ol-20-nicotinylhydrazone	12	1	7

[a] Data from Kincl and Dorfman (1963).

Table XXI presents a group of pregnane derivatives which showed shallow dose response curves, and ranges in relative potency of the order of tenfold, depending on the dose level studied (Kincl and Dorfman, 1963).

Other investigators reported on the activity of a variety of progesterone derivatives. Pincus (1956) found the following steroids to be less active than progesterone: 17α-hydroxyprogesterone, *D*-homoprogesterone, progesterone 3-monoenolacetate, 16α-methylnitrite of progesterone, 16-bromoprogesterone, 19-norprogesterone, 20β-hydroxypregn-4-en-3-one, *D*-homo-5β-pregnane-3,20-dione, 3β-hydroxy-pregn-5-ene-7,20-dione, 17α-ethynyltestosterone, 11β-hydroxy-17α-ethynyl-17β-acetoxyandrost-4-en-3-one, and 4-hydroxyprogesterone. 17α-Methylprogesterone was more active than progesterone.

Pincus and Chang (1953) found the following compounds inactive when injected at the 10 mg dose level: 17α-hydroxy-5α-pregnane-3,20-dione, 5α-pregnane-3β,17α-20-triol, 21-hydroxy-5α-pregnane-3,20-dione, 5β-pregnane-3,20-dione, 5β-pregnane-3α,20α-diol, 5β-pregnane-3α,20β-diol, pregna-4,16-diene-3,20-dione and 11α-hydroxyprogesterone.

TABLE XXII

ANTI-OVULATORY ACTIVITY OF VARIOUS STEROIDS[a]

Steroid	Minimum dose required to produce significant effect (mg): Subcutaneous route	Oral route
17α-Acetoxypregn-4-en-3,20-dione	0.08	10.0
6α-Methyl-17α-acetoxypregn-4-en-3,20-dione (XXII)	0.08	10.0
16α-Chloropregn-4-ene-3,20-dione	0.08	10.0
Pregna-3,5-diene-3,17α,20-triol triacetate	0.08	0.4
21-Fluoropregn-4-ene-3,20-dione	0.4	—
20-Thiopregn-4-en-3-one	1.0	> 10.0
17α-Acetoxypregna-4,6-diene-3,20-dione	2.0	—
Pregna-4,6-diene-3,11,20-trione	2.0	—
D-Homopregna-4,17(17α)-diene-3,20-dione	5.0	—
9α-Fluoro-17α-acetoxypregn-4-ene-3,11,20-trione	10.0	—
3β-Hydroxy-16α-methoxy-5β-pregnan-20-one	10.0	—
Progesterone	1.0	—

[a] Data from Pincus and Merrill (1961).

Pincus and Merrill (1961) have summarized their experience with a number of pregnane derivatives in the 1-day, precoital female rabbit. In this study, the minimum effective anti-ovulatory subcutaneous dose of progesterone was 1 mg in the rabbit. Orally, this steroid was active in the 5–10 mg range. Table XXII summarizes the data.

The following compounds studied by injection were judged to be inactive, or only marginally active (the numbers in parentheses indicate, in milligrams, the highest dose level studied):

16α,17α-Oxidopregn-4-ene-3,20-dione (2.0)
6β-Hydroxy-16α,17α-oxidopregn-4-ene-3,20-dione (10.0)
21-Acetoxypregn-4-ene-3,20-dione (2.0)
1α-Thioacetoxypregn-4-ene-3,20-dione (10.0)
1α,4α-Dithiopregnane-3,20-dione (10.0)
3β,17α-Acetoxypregn-5-ene-7,20-dione (10.0)
17α,19,21-Trihydroxypregn-4-ene-3,20-dione (10.0)
16β-Chloro-11β-acetoxy-17α-hydroxypregn-4-ene-3,20-dione (20.0)
1α-Thioacetoxy-17α-hydroxypregn-4-ene-3,20-dione (10.0)
20-Cyano-21-carboxypregna-4,17(20)-dien-3-one ethylesters (2.0)
22-Phenylpregna-4,21-dien-3-one (10.0)
22-[4-Methoxy]phenylpregna-4,21-dien-3-one (10.0)
17-Aza-21-chloro-*D*-homopregn-4-ene-3,20-dione (10.0)

2. *Oral Activity*

A number of pregnane derivatives was tested by Kincl and Dorfman (1963) for oral anti-ovulatory activity, using norethisterone (XX) as the standard compound (Table XXIII).

While 17α-acetoxyprogesterone was a very weak anti-ovulatory steroid, modifications of the structure by addition of methyl or halogen at the 6-position, with or without unsaturation greatly increased the activity. 6-Chloro-6^6-dehydro-17α-acetoxyprogesterone (XXIV) was the most active compound in this series, showing a relative potency of 1200 times that of the parent compound, 17α-acetoxyprogesterone. Compared to norethisterone at different portions of the dose-response curve, relative potencies of ten- to sixtyfold in potencies were found, with a mean value of 35. 6α-Methyl-17α-acetoxypregna-1,4-diene-3,20-dione had a relative activity of about 10 or greater. A similar high activity of about 9 (6–12) for 6-fluoro-17α-acetoxypregna-4,6-diene-3,20-dione was reported. Relative potency of 6-methyl-17α-acetoxypregna-4,6-diene-3,20-dione was rated as 5, while 17α-acetoxypregna-4,6-diene-3,20-dione was judged to have a relative potency greater than 3.

TABLE XXIII

RELATIVE ANTI-OVULATORY POTENCY OF 17α-ACETOXY PROGESTERONE DERIVATIVES ADMINISTERED BY GAVAGE[a]

Steroid	Number of rabbits used	Relative potency (norethisterone = 100)
6-Chloro-6-dehydro-17α-acetoxyprogesterone	33	3500
6α-Methyl-1-dehydro-17α-acetoxyprogesterone	17	$\geq$1000
6-Fluoro-6-dehydro-17α-acetoxyprogesterone	23	600–1200
6-Methyl-6-dehydro-17α-acetoxyprogesterone	9	500
6-Dehydro-17α-acetoxyprogesterone	13	300
6α-Methyl-17α-acetoxyprogesterone	14	260
6-Chloro-1,6-bisdehydro-17α-acetoxyprogesterone	2	$\geq$200
6α-Fluoro-16α-methyl-17α-acetoxyprogesterone	7	$\geq$125
6α-Chloro-17α-acetoxyprogesterone	15	60
6α-Fluoro-17α-acetoxyprogesterone	8	40–65
6α-Chloro-17α-acetoxy-1-dehydroprogesterone	21	30
17α-Acetoxy-19-norprogesterone	21	25
6α-Bromo-17α-acetoxyprogesterone	4	15
4-Chloro-17α-hydroxyprogesterone	13	12
16β-Chloro-17α-acetoxyprogesterone	3	10
4-Chloro-17α-acetoxyprogesterone	30	4
16α-Methyl-17α-acetoxyprogesterone	2	3
17α-Acetoxyprogesterone	2	3
17α-Acetoxy-1-dehydroprogesterone	5	2

[a] Data from Kincl and Dorfman (1963).

TABLE XXIIIA

THE EFFECT OF VARIOUS SUBSTITUENTS ON THE ANTI-OVULATORY ACTIVITY OF 17α-ACETOXYPROGESTERONE DERIVATIVES[a] (ORAL ADMINISTRATION)

Substituent	Relative potency			
	Δ^{4}	$\Delta^{1,4}$	$\Delta^{1,6}$	$\Delta^{1,4,6}$
None	1	0.66	90	—
6-Fluoro	13–22	—	180–360	—
6-Chloro	20	10.0	1170	$\geq$66
6-Bromo	5	—	—	—
6-Methyl	78	$\geq$300.0	150	—

[a] Data from Kincl and Dorfman (1963).

Some less active anti-ovulatory steroids are reported in Table XXIV. These compounds vary in their relative potencies from 0.6 that of norethisterone to as little as 0.01 the potency of the standard. Two nonprogestational anti-ovulatory steroids were reported: 3β-chloro-16β-methylisopregn-5-en-20-one and 3β-chloropregn-5-en-20-one. Three other compounds lacking an oxygen function at C-3 (XXVII, XXIX, and XXX) were discovered to be potent anti-ovulatory steroids. These

TABLE XXIV

RELATIVE ANTI-OVULATORY POTENCY OF VARIOUS STEROIDS ADMINISTERED BY GAVAGE[a]

Steroid	Number of rabbits used	Relative potency (norethisterone = 100)
17α-Acetoxypregna-3,5-dien-20-one (XXX)	15	50
17α-Acetoxypregn-5-en-20-one (XXIX)	14	40
6β,16α-Dimethylprogesterone (XXVII)	26	20
6,16α-Dimethylpregna-4-diene-3,20-dione (XXVI)	13	20
3β-Chloro-16β-methylpregn-5-en-20-one	6	20
3β-Fluoro-17α-acetoxypregn-5-en-20-one (XXVIII)	16	10
16β-Carboxamide-3β-acetoxy-17α-pregn-5-en-20-one	9	10–50
6-Dehydroretroprogesterone	19	5–30
3β-Chloropregn-5-en-20-one	4	5
3β-Hydroxy-16α,17α-methylenepregn-5-en-20-one	3	2
19-Norprogesterone (XXIII)	20	1
3β-Acetoxypregn-5-en-20-one-16α,17α-pyrazole	3	1

[a] Data from Kincl and Dorfman (1963) and Dorfman and Kincl (1963).

compounds, however, were found to be orally active when tested in the McPhail test for progestational activity (Kincl and Folch-Pi, 1962).

Removal of the 10β-methyl angular group increased the potency, as compared to the parent steroid. 17α-Acetoxy-19-norprogesterone (17α-acetoxy-19-norpregn-4-ene-3,20-dione, XXV) was at least 25 times as active as 17α-acetoxyprogesterone. The activity of the latter compound was evaluated to be less than 3% that of norethisterone. 19-Norprogesterone was active when 30 mg was given orally; in contrast, 600 mg of progesterone was needed to inhibit ovulation. 6-Dehydroretroprogesterone (10-isopregna-4,6-diene-3,20-dione, XXXI) possessed

between 5 and 30% the activity of norethisterone. Several other compounds were reported as inactive. These include (figures in parentheses indicate, in milligrams the highest dose level studied):

17β-Hydroxypregna-4,6-diene-3,20-dione (12.5)
6-Chloro-17α-acetoxypregna-4,6-dien-20-one (2.0)
6α-Methyl-17α,21-diacetoxypregn-4-ene-3,20-dione (1.25)
17α-Acetoxyprogesterone (20.0)
10β-Chloro-19-norpregna-1,4-diene-3,20-dione (10.0)

B. Estrane Derivatives

In contrast to the high activity observed with estrogens in the parabiotic rat, ring A phenolic steroids are only moderately active in the rabbit. The derivatives of 19-nortestosterone are able, to varying degrees, to inhibit ovulation in the rabbit.

1. Ring A Phenolic Steroids

Pincus and Merrill (1961) found inhibition, following injection of estrone, 0.4–10.0 mg dose range, but estradiol at 10.5 mg was ineffective. 17α-Ethylestradiol-17β was fully effective at 10 mg dose level, and marginally effective at 0.4 and 2.0 mg. The activity of these steroids and other compounds studied is given in Table XXV.

TABLE XXV

Anti-Ovulatory Activity of Various Steroids[a]

Ring A phenolic steroid	Effective dose (mg)	
	Subcutaneous	Oral
Estradiol-17β	>0.1	>0.5
Estradiol-17β 3-methylether	10.0	-
Estrone	≧10.0	-
1-Hydroxyestradiol-17β 1,3,17-triacetate	≧10.0	—
17α-Ethylestradiol-17β	10.0	—
4-Methylestradiol-17β 3-methylether	≧10.0	—
17ξ-Thioestra-1,3,5(10)-triene-3-ol	10.0	—
17ξ-Thioestra-1,3,5(10)-triene-3-methoxy	≧10.0	—
3-Methoxy-21-hydroxy-24-carboxy-25-norchola-1,3,5(10)-trien-11-one-21,24 lactone	≧10.0	—

[a] Data from Pincus and Merrill (1961).

17α-Ethynylestradiol 3-methylether (XVI), at a dose of 0.2 mg by subcutaneous injection was active, but 1.0 mg by gavage was inactive. 10β-Chloro-17β-hydroxyestra-1,4-dien-3-one, 2.0 mg given by injection and 5.0 orally, was inactive (Kincl and Dorfman, 1963).

2. *Δ⁴-Estrane (19-Nortestosterone) Derivatives*

Pincus (1956) reported norethisterone (XX) to be four times as active as progesterone when injected and also active when given orally. Norethynodrel (XIX) was judged to be active by mouth and ten times as active as progesterone by injection. Data on these and other 17α-allyl substituted compounds, both Δ^4 derivatives, and some $\Delta^{5(10)}$ analogs are given in Table XXVI. The acetate of compound XVII, 17α-ethyl-

TABLE XXVI

ANTI-OVULATORY ACTIVITY OF VARIOUS 19-NORTESTOSTERONE DERIVATIVES[a]

17α-Alkyl substituents	Minimal dose (mg) needed to produce inhibition of ovulation			
	Δ^4 derivatives		$\Delta^{5(10)}$ derivatives	
	Subcutaneous	Oral	Subcutaneous	Oral
No Substituent	--	—	2.0	—
$—CH_3$ (XVIII)	0.5	5.0	—	—
$—CH_2CH_3$ (XVII)	5.0	5.0	2.0	—
$—CH_2CH_2CH_3$	0.2	≧ 10.0	10.0	—
$—C{\equiv}CH$ (XX)	0.5	0.5	10.0	1.0 (XIX)
$—C{\equiv}CCH_3$	2.0	≧ 10.0	—	—
$—C{\equiv}CCH_2CH_3$	10.0	--	—	—
$—CH_2C(CH_3){=}CH_2$	0.1	> 5.0	—	-
$—CH_2CH{=}CH_2$	0.1	10.0	·	—
$—CH_2CH{=}CHCH_3$	0.04	—	> 2.0	—
$—CH_2CH_2OH$	≧ 10.0	--	—	—

[a] Data from Pincus and Merrill (1961).

17β-acetoxyestr-4-en-3-one, was reported to be only marginally active at a dose of 10 mg (subcutaneous route). The enol acetate of compound XX, 17α-ethynylestra-3,5-diene-3,17β-diol diacetate was found fully active at approximately the same dose as norethisterone (0.4 mg) when given subcutaneously. 17α-Ethynyl-17β-acetoxyestra-4,6-dien-3-one, and 16α-methyl-16β,17β-dihydroxyestr-4-en-3-one were active at 10 mg dose level (subcutaneous administration). Two other 19-noretestosterone derivatives were reported to be only marginally active, when

tested by subcutaneous injection, 10 mg dose level: 4,4-diacetoxy-17β-hydroxyestra-1,5(10)-dien-3-one; 17-methyl-17-propyl-18,19-bis norestra-4,13(18)-dien-3-one.

Elton and Edgren (1958) reported 17α-2-methallyl-19-nortestosterone effective at 1.0 mg dose level when tested by subcutaneous injection. In their test, progesterone was fully active at the same dose.

Kincl and Dorfman (1963) found the effective dose of norethisterone (XX) to be 0.15 mg by subcutaneous injection and 0.625 mg by the oral route. The same authors (Dorfman and Kincl, 1963) observed

TABLE XXVII

RELATIVE POTENCY OF VARIOUS STEROIDS ASSAYED ORALLY FOR INHIBITION OF OVULATION IN THE RABBIT[a]

Compound	Number of rabbits	Relative potency
Norethisterone (XX)	—	100
17α-Ethylestr-4-en-17β-ol (XXXII)	27	100
17α-Ethynylestr-4-en-17β-ol (XXXIII)	18	100
17α-Methyl-19-nortestosterone (XVIII)	30	100
17α-Vinyl-19-nortestosterone	30	40
17α-Allylestr-4-en-17β-ol	17	33
Norethynodrel (XIX)	41	>25
17α-Ethynyl-17β-hydroxy-5α-estran-3-one	16	12
4-Chloro-17α-ethynyl-17β-hydroxyestr-4-en-3-one	8	>6
17α-Ethynyl-5α-estrane-3β,17β-diol	11	>5

[a] Data from Dorfman and Kincl (1963).

the relative activity of several 19-nortestosterone, and 3-deoxy derivatives, following oral administration (Table XXVII). Two 3-deoxy 19-nortestosterone derivatives, ethylestrenol (17α-ethylestr-4-en-17β-ol, XXXII) and 3-deoxy norethisterone (XXXIII) were found to be as active as norethisterone. A dose of 2.5 mg of norethynodrel (XIX) was observed to be insufficient in blocking ovulation, and this compound was judged to be less than one-fourth as active as norethisterone.

C. ANDROSTANE DERIVATIVES

Only a few androstane derivatives, apart from 19-nortestosterone compounds, described in Section IV, B, have been studied. The activity of several compounds is reported: injected compounds, in Table XXVIII; and compounds given by the oral route, in Table XXIX

TABLE XXVIII

RELATIVE ANTI-OVULATORY ACTIVITY OF VARIOUS SUBCUTANEOUSLY ADMINISTERED STEROIDS[a]

Steroid	Number of rabbits	Relative potency (norethisterone = 100)
2α-Hydroxymethyl-17β-hydroxyandrostan-3-one	6	14
6α-Chloro-17α-ethynyl-17β-hydroxyandrost-4-en-3-one	3	4
2-Hydroxymethyl-17α-methyl-17β-hydroxy-5α-androstan-3-one	5	4
16-Dimethylaminomethylen-androstan-3β-ol 17-one	4	1
16-Methylene-*N*-piperidyl-4-androstane-3,17 dione	8	1
17α-Methyl-5α-androst-2-en-17β-ol	15	5-100
3β-Hydroxy-5α-androstano-16,17-pyrazole	5	1-10

[a] Data from Kincl and Dorfman (1963)

TABLE XXIX

RELATIVE ANTI-OVULATORY ACTIVITY OF VARIOUS ORALLY ADMINISTERED STEROIDS[a]

Steroid	Number of rabbits	Relative potency (norethisterone = 100)
2-Hydroxymethylene-17α-methyl-17β-hydroxy-5α-androstan-3-one	18	<5
2α-Hydroxymethyl-17α-methyl-17β-hydroxy-5α-androstan-3-one (XIII)	29	≧200
2-Methylene-*N*-ethylenediethylamino-17α-methyl-17β-hydroxy-5α-androstan-3-one	8	15
3β-Hydroxy-5α-androstano-16,17-pyrazole	18	20
17α-Ethynyl-5α-androst-2-en-17β-ol	4	7

[a] Data from Kincl and Dorfman (1963).

(Kincl and Dorfman, 1963). One compound, 2α-hydroxymethyl-17α-methyl-17β-hydroxy-5α-androstan-3-one (XIII), was noted to be at least twice as active as norethisterone; the closely related 2-hydroxymethylene derivative which differs only by the presence of an exocyclic double bond at C-2 was judged to possess less than 2% the activity of compound XIII.

Other androstane derivatives studied by subcutaneous injection were reported by Pincus and Merrill (1961) to be marginally active at a 10 mg dose level as follows:

19-Acetoxyandrosta-1,4-diene-3,17-dione
17α-Methyl-2,17β-dihydroxyandrosta-1,4-dien-3-one
16β-Iodo-3β-hydroxyandrost-5-en-17-one
17β-[5-(3,4-Dihydro-1,3-diazine)]androst-4-en-3-one
1β,3β,5β,10,14β-Pentahydroxy-25-norchol-20(21)-en-24-oic acid 21,24-lactone

V. Miscellaneous Methods

A. Activity Assayed in the Intact Animal

This section deals with selected reference to the inhibition of gonadal development by steroid treatment in the intact animal. An excellent historical review is available (Burrows, 1949).

1. Inhibition in the Immature Animal

Biddulph (1939) treated newborn rats by injection, with a daily dose of 10 μg of testosterone propionate, from birth to 31 days of age; this treatment produced testicular atrophy, and mature sperm was absent. Continuous treatment from birth to 51 or to 81 days of age produced less severe testicular atrophy, but apparently did not inhibit spermatogenesis, and mature sperm was present in the testes.

Wilson and Wilson (1943) studied the effect of testosterone propionate on fecundity, sexual potency, and libido in male rats. The steroid was given by subcutaneous injection at varying dose levels three times a week for 28 days, starting treatment at birth and at 5, 10, 15, and 20 days of age and subjecting the animals to the three tests at ages ranging from 125 to 190 days.

Testosterone treatment, 0.125 mg per injection, from birth to 28 days of age rendered 7 out of 8 males infertile, sexually impotent, and with decreased libido. At autopsy, testes, ventral prostate, and seminal vesicle weights were markedly decreased. If the treatment was initiated at 5 days of age or later (3.0 mg per injection), most of the males were fertile and the inhibition of sexual potency was less marked.

A single subcutaneous injection of testosterone propionate, 1.25 mg given to 5-day-old mice (Barraclough and Leathem, 1954) and rats (Barraclough and Gorski, 1961) produced sterile adult animals with persistent cornification of the vaginal epithelium and polycystic ovaries. Similar treatment with progesterone (3.0 mg), 6α-methyl-17α-acetoxy-

progesterone, and cortisone is not (1.0 mg) effective (Shipley and Meyer, 1962). Continuous administration of 1 mg testosterone propionate from birth to 30 days of age, with an additional month before autopsy, produced marked uterine and ovarian atrophy (Selye, 1940). A single injection of estradiol benzoate and of testosterone propionate, 250 μg, given either singly or in combination to 22-day-old rat, mouse, hamster, and guinea pig produced testicular atrophy in all four species, sacrificed 72 hours after the treatment (Leathem and Wolf, 1955).

In the newborn male rat, a single injection of estradiol-17β benzoate, at 120 μg, produced inhibition of spermatogenesis and decrease in the weight of the accessory sex glands of animals examined at the age of 140 days. The treatment was effective in the 5-day-old animals, but not if given to animals at the age of 10 days or later (Kincl *et al.*, 1963).

In 10-day-old mice a single injection of testosterone propionate, 1 mg, resulted in testicular atrophy and delay of spermatogenesis, which was recovered by 30 days post-injection.

In the 22- to 32-day-old rat a daily administration of 20 and 50 μg of testosterone propionate for 10 days produced testicular atrophy (Rubinstein and Kurland, 1941). Shipley (1962), Beyler and Potts (1957), and Saunders and Drill (1958) investigated the activity of various synthetic steroids, given in graded doses by subcutaneous injection to immature rats of 21–25 days of age. Table XXX lists some typical studies on the effective doses of certain steroids needed to produce testicular atrophy.

TABLE XXX

EFFECT OF VARIOUS STEROIDS ADMINISTERED SUBCUTANEOUSLY TO INTACT, IMMATURE RATS

Compound	Daily dose (μg)	Duration of treatment	Decrease in testes weight (%)	References[a]
Testosterone	25	14	30	1
Testosterone propionate	12.5	14	42	1
19-Nortestosterone		14	54	1
19-Nortestosterone propionate	12.5	14	63	1
17α-Ethyl-19-nortestosterone (XVII)	500/kg	30	89	2

[a] Key to References: 1. Shipley (1962). 2. Saunders and Drill (1958).

Two compounds, 19-nortestosterone and its propionate, were effective at doses that produced minimal stimulation of ventral prostate and seminal vesicle growth, indicating the ability of these two compounds to inhibit gonadotropin secretion, in spite of reduced androgenicity. The most marked was the effect of norethynodrel (XVII). This compound not only produced marked testicular atrophy at the highest dose studied (100 μg/kg dose produced only marginal effect), but also a decrease in body, ventral prostate, and seminal vesicle weights. In the intact female this compound produced significant decrease in ovarian weight at 100 and 500 μg/kg dose level.

The activity of methyltestosterone and four 19-nortestosterone derivatives, following oral administration of 1.0 mg daily, for 7, 21, and 42 days was reported by McGinty and Djerassi (1958). At the dose level studied, 17α-methyltestosterone and the 19-nor analog (XVIII) were found to be less active, but more androgenic, whereas ethynyl (XIX) and 17α-vinyl-19-nortestosterone derivatives were the most active; these two compounds also apparently prevented the testicular descent, if given for 7 and 21 days, respectively. The activity of 17α-ethyl-19-nortestosterone (XVII) was judged to be somewhat less than for the former compounds.

Purshottam *et al.* (1961) used 20-day-old Swiss mice in which ovulation was stimulated with an intraperitoneal injection of pregnant mare serum (PMS), 2 IU, followed by a similar injection of human chorionic gonadotropin (HCG), 1 IU, given 42–43 hours later. The presence, or absence, of ova was determined in the oviducts at autopsy carried out 20 hours after the HCG injection. A variety of nonsteroidal compounds and a few steroids were studied for inhibition of ovulation; the test material was administered presumably before the HCG injections. Of the eight steroids tested, only progesterone (0.5 mg dose) and 17α-ethynyl-17β-hydroxyestr-5(10)-en-3-one (1.5 mg dose) produced significant inhibition of ovulation. Estrone (1.0 mg), 17α-ethynylestradiol-17β 3-methylether (0.05 mg), cortisone (3.0 mg), 17α-ethyl-19-nortestosterone (0.5 mg), 17α-methallyl-19-nortestosterone (1.5 mg), and 6α-chloro-11-acetoxyprogesterone (2.0 mg) were found to inhibit ovulation only moderately at the dose levels studied.

2. *Inhibition in the Mature Animal*

Activity of subcutaneously administered steroids in inhibiting pituitary function, as judged by gonadal atrophy in the male and female rat, has been studied by Lerner *et al.* (1959) for testosterone propionate and 19-nortestosterone benzoate; Beyler and Potts (1957) for estradiol-17β, estradiol-17β benzoate, estrone, diethylstilbestrol dipal-

mitate, and 17α-ethynyl-3β,17β-dihydroxyandrost-5-en-3-cyclohexyl propionate; Saunders and Drill (1958) for 17α-ethyl-19-nortestosterone. McGinty and Djerassi (1958) studied the activity of methyltestosterone and of 17α-ethynyl-, 17α-ethyl-, 17α-vinyl-, and 17α-methyl-19-nortestosterone, administered orally.

In general, the amounts needed by various investigators to produce inhibition of the pituitary function in the mature animal did not differ markedly from the amounts required for this effect in the immature, 21- to 25-day-old rat. The suitability of adult mice, hamsters, guinea pigs, and rabbits for measuring gonadotropin inhibition was demonstrated by Leatham and Wolf (1955).

A high dose of cortisol, 25 mg per day, was reported to produce testicular atrophy, decrease in interstitial material, and hypotrophy of seminal vesicles. This change was reversible (Weller, 1962).

The pituitary-inhibiting effect of testosterone, 2 mg, and of 17α-methyl-17β-hydroxyandrosta-1,4-dien-3-one, 0.2 and 2.0 mg, given by subcutaneous injection for 14 days was described by Cohnen (1962), who measured the volume of cell nuclei in Leydig cells and ovarian interstitial cells. Sulman (1960) described the effect of testosterone propionate, heptanoate, and undecylate following protracted therapy.

B. Inhibition of Spermatogenesis

Ludwig (1950) examined the effect of testosterone propionate and estradiol-17β benzoate on spermatogenesis and examined at the same time the pituitaries of the treated animals for gonadotropin potency by assaying in the immature female rats. He used 30-day-old rats and administered the test substance by daily injection for 30 days. A daily dose of 0.1 mg was required to suppress spermatogenesis; the weight of the testes was reduced to 35% and the gonadotropin content to 36% of the control values. Larger doses, 1.0 and 3.0 mg, reduced the gonadotropin potency to 7% and 0%, respectively; however, the testes weights were decreased by 16% and 11% only, due to the direct stimulating effect. Addition of larger amounts (1.0 mg) of testosterone propionate was found to prevent inhibition of spermatogenesis, produced by 8.4 μg of estradiol-17β benzoate. Direct implantation of a 5% and 15% testosterone propionate pellet into the testes likewise protected the animal receiving 0.1 mg of testosterone propionate subcutaneously against inhibition of spermatogenesis.

Kincl *et al.* (1965) studied the inhibition of spermatogenesis in the rat using various androgens. Intact males, 21 days old, were injected subcutaneously with the test substance once daily for 21 days. Autopsy

was performed the day following the last administration. At autopsy, testicular, ventral prostate, seminal vesicle, and levator ani muscle weights were taken. The testicular tissue was examined histologically to judge the extent of inhibition of spermatogenesis. Table XXXI summarizes the results.

TABLE XXXI

THE EFFECT OF VARIOUS STEROIDS ON INHIBITION OF SPERMATOGENESIS IN THE RAT

Compound administered	Number of rats	Daily dose (μg)	Organ weights (mg ± SE) Testes	Ventral prostate	Seminal vesicles	Levator ani
None	15	0	2021 ± 35.0	107.0 ± 4.0	71.3 ± 3.1	91.3 ± 5.0
Testosterone	5	40	1910 ± 58 5	134.0 ± 16.0	91.4 ± 7.0	101.0 ± 10.0
	5	100	1510 ± 78.3	112.0 ± 8.2	76.2 ± 5.3	98.1 ± 6.3
	15	200	1109 ± 45.0	171.0 ± 7.0	144.0 ± 5.0	129.0 ± 4.0
2α-Hydroxymethyl-17β-hydroxy-5α-androstan-3-one	5	40	1850 ± 166.0	99.5 ± 13.0	59.8 ± 7.3	94.5 ± 6.9
	5	200	908 ± 78.0	69.4 ± 7.7	38.3 ± 3.5	116.0 ± 10.0
2-Hydroxymethyl-androst-2-en-17β-ol	5	40	1726 ± 94.0	79.8 ± 5 9	40.8 ± 3.8	112.0 ± 6.1
	5	200	601 ± 24 0	32 4 ± 1 6	30.7 ± 1.5	147.0 ± 3.9
2,17α-Dimethyl-androst-2-en-17β-ol	5	67	1312 ± 248 0	63 5 ± 9 6	41.8 ± 7.6	93.3 ± 9.4
	5	200	723 ± 24 0	67 9 ± 4.6	50 2 ± 1.3	129.0 ± 5.0
2-Formylandrost-2-en-17β-ol	5	40	1780 ± 63 0	84.3 ± 5.7	68.1 ± 6.8	108.0 ± 4.8
	5	200	546 ± 37.6	31.0 ± 2.3	23.6 ± 1 2	141.0 ± 6.1

Animals treated with a low dose of testosterone, 40 μg and 100 μg per day, and with the low dose of 2α-hydroxymethyldihydrotestosterone (V), 2-hydroxymethylandrost-2-en-17β-ol, and 2-formylandrost-2-en-17β-ol, had normally developed testes, and spermatogenesis was not arrested. A daily dose of 40 μg of 2,17α-dimethylandrost-2-en-17β-ol, and 200 μg of the other steroids studied, produced a decrease in seminiferous tubule size, and arrest of spermatogenesis, mostly of the secondary spermatocyte stage. The interstitial tissue was decreased. At the highest dose level studied, testosterone treatment produced marked stimulation in the growth of the accessory sex organs. The effective dose

of the four synthetic androgens did not influence the growth of the ventral prostate and seminal vesicles, indicating a separation between gonadotropin inhibition and androgenic potency.

The pituitary activity was fully recovered after a 30-day period, and histologically the testes appeared normal, although the gonadal and sex tissue weights had not been recovered (Table XXXII).

TABLE XXXII

INHIBITION OF SPERMATOGENESIS AND RECOVERY OF MALE RATS TREATED BY SUBCUTANEOUS INJECTION FOR 30 DAYS[a]

Treatment	Body weight (gm)	Organ weights (mg ± SE): Testes	Ventral prostate	Seminal vesicles
Autopsy—1 day post-treatment				
Control	172	1857 ± 70.3	97.9 ± 5.5	61 1 ± 4.2
Testosterone	190	1025 ± 96.5	165 6 ± 11.3	135.6 ± 10.1
2-Hydroxymethylandrost-2-en-17β-ol	163	676 ± 46.5	65.8 ± 12.7	41 2 ± 4 2
Autopsy—30 days post-treatment				
Control	293	3178 ± 58 7	350 8 ± 14.6	266.2 ± 17.7
Testosterone	318	2972 ± 50.0	324.1 ± 16.6	273.5 ± 5.5
2-Hydroxymethylandrost-2-en-17β ol	264	2417 ± 266 0	225 6 ± 49.2	217.9 ± 42.0
Autopsy—60 days post-treatment				
Control	331	3392 ± 105 0	512 0 ± 36 0	352.0 ± 18 5
Testosterone	405	3514 ± 45 8	520.0 ± 38.0	387.0 ± 11.1
2-Hydroxymethylandrost-2-en-17β-ol	397	3388 ± 27 0	365.0 ± 33.4	313 0 ± 15 4

[a] Six animals per group; 200 μg per day.

Patanelli and Nelson (1959) studied the effect of five synthetic progestational agents, testosterone propionate, and an estrogen on inhibition of spermatogenesis in 30-day-old rats. The compounds were administered either subcutaneously or orally for a period of 27–30 days. At autopsy, the gonads, sex organs, adrenals, pituitary, thymus, and kidney weights were recorded; the extent of inhibition of spermatogenesis was judged microscopically. The pituitaries were assayed for FSH and LH content. Tables XXXIIA and XXXIIB record the results.

TABLE XXXIIA

INHIBITION OF SPERMATOGENESIS IN THE RAT WITH VARIOUS STEROIDS[a]
(SUBCUTANEOUS INJECTION)

Steroid	Dose (mg)	Organ weights (mg): Testes	Seminal vesicles	Ventral prostate
None	0	2916	277	244
17-Ethynyl-17β-hydroxy-estr-5(10)-en-3-one (XIX)	0.25	801	52.9	35.9
	0.5	849	46.8	33.2
	0.75	447	35.0	22.5
	1.0	361	36.1	16.5
None	0	2845	241	225
17α-Ethynyl-19-nortestosterone (XX)	0.25	1636	93.1	67.1
	0.5	949	45.2	37.6
	0.75	796	48.7	61.2
	1.0	484	54.0	41.9
17α-Ethyl-19-nortestosterone (XVII)	0.5	1712	68.0	87.0
	1.0	1382	100.0	78.9
	2.5	2019	159.0	132.0
	4.0	2003	291.0	182.0
None	0	3521	270	255
17α-Ethynylestradiol 3-methylether (XVI)	0.01	429	47.7	19.0
	0.02	451	55.2	19.7

[a] Data from Patanelli and Nelson (1959).

The inhibitory effect of 17α-ethynyl-19-nortestosterone (XX) was found to be less than that of the $\Delta^{5(10)}$ analog XIX, and 1.0 mg by injection and 2.0 mg orally was required for complete inhibition. 17α-Ethyl-19-nortestosterone did not inhibit spermatogenesis completely at the doses given. Two other compounds, 17α-hydroxyprogesterone acetate and caproate, given at doses of 0.5–2.5 mg and of 6.25 and 25.0 mg, respectively, failed to suppress pituitary secretion of gonadotropin at the doses studied.

TABLE XXXIIB

INHIBITION OF SPERMATOGENESIS IN THE RAT WITH VARIOUS STEROIDS[a] (ORAL ADMINISTRATION)

Steroid	Dose (mg)	Organ weights (mg) Testes	Seminal vesicles	Ventral prostate
None	0	2916	277	244
17α-Ethynyl-17β-hydroxyestr-5(10)-en-3-one (XIX)	0.5	1636	92.0	80.3
	1.0	966	36 7	26.8
	1.5	365	27.7	12.5
None	0	2845	241	225
17α-Ethynyl-19-nortestosterone (XX)	1.0	2005	157.0	151.0
	1.5	1672	146 0	90.5
	2.0	371	23.6	17.3
17α-Ethyl-19-nortestosterone (XVII)	1.0	2647	180.0	149.0
	2 5	1505	70 5	54.5
	4.0	1672	108.0	74.5
None		2797	275	274
17α-Ethynylestradiol-17β 3-methylether (XVI)	0.001	2880	228.0	164
	0.005	1991	140.0	84.0
	0.01	1479	95.2	69.2
	0.02	552	26 2	21.5
	0.05	348	30.2	15.8
	0.2	367	38.0	13.9

[a] Data from Patanelli and Nelson (1959).

C. ANTI-LUTEINIZING ACTIVITY

A daily dose of 0.25–0.5 mg of progesterone, administered by subcutaneous injection for 4–10 days to immature female rats, receiving also purified FSH, inhibited luteinization (Astwood and Fevold, 1939).

In pregnant mice, a daily injection of testosterone propionate, 0.25 mg per day, for the first 4–10 days of pregnancy, caused regression and resorption of corpora lutea (Burdick and Emerson, 1939). Inhibition of the luteinization in the immature rat by progestational agents was

studied by Junkmann (1957) and Shipley (1962). Junkmann used young rats which received a single injection of estradiol-17β valerate, 10 μg. A dose of 10 mg daily for 7 days was sufficient to produce an almost complete inhibition of luteinization. Rats receiving a dose of 1 mg per day produced an average of 0.9 corpus luteum. A single injection of 17α-hydroxyprogesterone caproate, 10 mg, was also fully effective (average 0.2 corpus luteum per rat). Two other long-acting esters of 17α-hydroxyprogesterone were studied by Gleason and Parker (1959) and by Shipley (1962), who finds it advantageous to use a 35-day-old rat without the estrogen priming. Results obtained are presented in Table XXXIII.

TABLE XXXIII

ANTI-LUTEINIZING ACTIVITY OF VARIOUS SUBCUTANEOUSLY ADMINISTERED STEROIDS

Steroid	Dose range studied (mg)	Effective daily dose (mg)	References[c]
Progesterone	1.0–10.0	1.0	1
Progesterone	0.125–2.0	0.5–1.0	3
17α-Hydroxyprogesterone caproate		10.0[a]	1
17α-Hydroxyprogesterone heptanoate		10.0[b]	2
3-Hydrazone-17α-heptanoxy-pregn-4-en-30-one		10.0[b]	2
6α-Methyl-17α-acetoxypregn-4-ene-3,20-dione	0.025–0.40	0.1–0.2	3
17α-Acetoxyprogesterone		0.4–0.5	
17α-Ethynyl-19-nortestosterone		0.2–0.4	
Pregna-4,9(11)-diene-3,20-dione		0.2–0.3	

[a] Given in a single injection.
[b] Single subcutaneous injection equivalent to 10 mg progesterone.
[c] Key to References:
1. Junkmann (1957). 2. Gleason and Parker (1359). 3. Shipley (1962).

Lipschutz and Figueroa (1957) also used intrasplenic ovarian autografts in the castrated guinea pig to study the anti-luteinizing activity of progesterone and other steroids. The test compounds (in pellet form), either alone, or in admixture with cholesterol, were implanted subcutaneously 2 weeks after castration; the test animals were sacrificed 45 days later. The ovarian autograft was examined histologically for the

presence, or absence, of corpora lutea. A pellet of estradiol-17β was implanted at the same time as the test substance, since it was observed that the autograft failed to exhibit corpora lutea in the absence of estradiol-17β (Mardones *et al.*, 1951). The activity of progesterone, and other steroids, is given in Table XXXIV.

Several other compounds were found to be inactive at the dose level indicated (Figueroa and Lipschutz, 1957); 17α-hydroxyprogesterone

TABLE XXXIV

ANTI-FERTILITY ACTIVITY OF VARIOUS STEROID IMPLANTS IN THE GUINEA PIG

Steroid	Average daily absorption (μg)	Number of animals	Number of animals with corpora lutea	References[a]
Progesterone	12	18	9	1
	20	10	3	1
19-Norprogesterone	3	9	1	2
	19	9	0	2
11-Dehydroprogesterone	11	9	3	2
	42	11	0	2
11-Ketoprogesterone	41	6	2	2
	560	8	0	2
11β-Hydroxyprogesterone	47	9	1	2
11α-Hydroxyprogesterone	43	19	14	2
9α-Fluoro-11β-hydroxy-progesterone	3	6	2	3
	10	6	0	3
	12	8	0	3
9α-Bromo-11β-keto-progesterone	15	10	2	3
	25	5	1	3
	38	5	0	3
17α-Ethynyltestosterone	173	10	10	1
	728	4	3	1
17α-Vinyltestosterone	75–244	11	8	1
	352–659	6	3	1
17α-Ethynylandrost-5-en-3β,17β-diol	82–286	7	7	1
17α-Ethynyl-19-nortesto-sterone	113	6	2	3
Deoxycorticosterone	8–43	7	3	1
	176–260	15	3	1
	283–439	3	0	1

[a] Key to References:
1. Mardones *et al.* (1951). 2. Mardones *et al.* (1956). 3. Lipschutz and Figueroa (1957).

(277 μg); 17α-hydroxyprogesterone caproate (213 μg); 17α,21-dihydroxypregn-4-ene-3,20-dione (427 μg); cortisone acetate (1742 μg); cortisol acetate (1600 μg); and cortisol (5140 μg).

D. Anti-Fertility Tests

1. *Female Rats and Mice*

Lipschutz and Iglesias (1961) used pellets of 19-norprogesterone implanted subcutaneously in the dorsal region of 65-day-old female mice kept with untreated mates, and measured the fertility index during the period of implantation. The pellets, weighing approximately 30 mg, usually were made with 20–40% of the tested compound mixed with 60–80% of cholesterol. At the end of the test period the implants were extracted and the daily absorption was calculated by dividing the differences in weight by the number of days during which the pellets were implanted.

The absorption rate of 19-norprogesterone (XXIII) was calculated at 2–4 μg per day with a 20% pellet and at 12–16 μg with a 40% pellet. At both concentrations, the females remained sterile during the period of observation and fully recovered fertility after extraction in 20–45 days. It was assumed that the implant inhibited the secretion of the luteinizing hormone, since corpora lutea were absent in animals so treated.

Daily treatment by subcutaneous injection of female rats given testosterone (2 mg), produced sterility and anestrus, but following cessation of treatment the reproductive ability returned to normal (Huffman, 1941). The activity of various steroids following daily treatment by subcutaneous injection for 35-day to 90-day-old female rats has been studied by Saunders and Drill (1958) (Table XXXV), who also measured the recovery of fertility upon cessation of treatment. Estrone was found to be the most active; at a daily dose of 0.01 mg, administered in oil solution, it was found to be 100% effective, and 71% of the animals so treated recovered fertility in the first 30-day post-treatment period.

A 16-methyl substituted estrogen (3-methoxy-16α-methylestra-1,3,-5(10)-triene-16β,17β-diol, XXXIV) was found to be only weakly active, and a thousandfold dose was required for complete inhibition. Testosterone propionate and various 17α-alkyl-19-nortestosterone derivatives were active. The daily dose needed to produce infertility varied from 0.2 mg for norethynodrel (XIX) to 2.0 for 17α-(1-methalkyl-19-nortestosterone), and 70–90% of the animals recovered fertility in the 30-

TABLE XXXV

EFFECT OF VARIOUS SUBCUTANEOUSLY ADMINISTERED STEROIDS ON FERTILITY IN RATS[a]

Compound	Range studied (mg/kg)	Daily dose needed to produce 100% infertility (mg/kg)	% Fertile within 30 days after cessation of treatment
Estrone	0.005–0.1	0.01	71
17α-Ethynyl-17β-hydroxy-estr-5(10)-en-3-one (XIX)	0.1–5.0	0.2	90
Testosterone propionate	0.1–1.0	0.5	67
17α-Ethyl-19-nortestosterone (XVII)	0.005–5.0	1.0	80
17α-(2-Methallyl)-19-nortestosterone	0.1–1.0	1.0	90
17α-(1-Methallyl)-19-nortestosterone	0.2–2.0	2.0	89
17α-Methyl-17β-hydroxy-5α-estran-3-one	5.0	5.0	70
Progesterone	0.2–5.0	5.0	0

[a] Data from Saunders and Drill (1958).

day, post-treatment period. Progesterone was weakly active, 5.0 mg being needed, but recovery was slow. None of the ten animals became pregnant within the first 30 days; four animals became gravid in the 31- to 60-day period and four animals in the 61- to 150-day period. However, two of the animals did not recover fertility even after 150 days post-treatment. Dorfman and Kincl (1963) studied the activity of various steroids, employing essentially the method as described by Lednicer *et al.* (1961). Adult female rats in the proestrus stage of the cycle were used. Each female was caged with a male of proved fertility for 48 hours; successful mating was established by taking vaginal smears after insemination was established the first day. The rats were treated by subcutaneous injection for 7 days, and autopsy was done on the ninth day of the test. The steroids were suspended in carboxymethylcellulose medium. The results of the experiment are given in Table XXXVI.

Falconi *et al.* (1961) employed the method used by Saunders and Drill (1958) comparing the activity of testosterone propionate, progesterone, progesterone 3-cyclopentyl enolether given subcutaneously, and of 17α-acetoxyprogesterone and 6α-methyl-17α-acetoxyproges-

TABLE XXXVI

ANTI-FERTILITY ACTIVITY OF VARIOUS STEROIDS IN RATS (SUBCUTANEOUS INJECTION)

Compound	Daily dose (μg)	Number of animals	Average number of implant sites	% Inhibition
3-Methoxy-17α-ethynylestra-1,3,5(10)-trien-17β-ol	1.0	3	9.0	33
	5.0	3	2.3	67
	25.0	3	0.0	100
19-Norprogesterone	100.0	3	11.0	0
	1000.0	3	4.0	67
2-Hydroxymethylandrost-2-en-17β-ol	3000.0	3	4.0	67
	10000.0	6	0.0	100
3-Methoxy-17α-19-norpregna-1,3,5(10)-trien-17β-ol acetate	100.0	6	6.0	50
	500.0	3	0.0	100
	900 0	3	0.0	100
	1000.0	9	1.0	92
	3000.0	3	0 0	100
	5000.0	3	0.0	100
	10000 0	3	0.0	100

terone, both given orally. A daily subcutaneous injection of testosterone propionate, 0.344 mg, and oral administration, 0.193 mg, of 6α-methyl-17α-acetoxyprogesterone inhibited mating and estrus in the majority of animals; the animals that mated were sterile. The other compounds which were administered at 1–2 μM dose levels did not inhibit estrus, mating, or fertility. The oral activity of six additional steroids was reported by Falconi and Ercoli (1961). The compounds included in this study were 17α-ethynyl-19-nortestosterone acetate, 17β-3-cyclopentyl enolether, and the cyclopentyl enolethers of 17α-acetoxyprogesterone and 6α-methyl-17α-acetoxyprogesterone. The last two parent compounds mentioned, and progesterone, were included for comparative purposes. The effect on the inhibition of estrus, mating, and fertilization was studied. The authors stated that whereas the enol formation in the case of 17α-acetoxyprogesterone and of the 6α-methyl derivative decreased the inhibitory effect, norethisterone acetate enolether was a potent anti-fertility agent, although it did not disturb sexual receptivity or cause permanent sterility. The effective dose of norethisterone acetate was 1.362 mg/day; the 3-cyclopentyl enolether was active at a lower dose, i.e., 0.409 mg.

Holmes and Mandl (1962) studied the effect of 17α-ethynyl-17β-hydroxyestr-5(10)-en-3-one (XIX) given by subcutaneous injection (0.2 mg and 1.0 mg per day) for 48 days to female rats, 107–142 days of age. The treatment caused sterility, but did not lead consistently to inhibition of ovulation. The ovaries of some animals contained a variable number of corpora lutea at different stages of development and regression. On the other hand, typical and regular estrous cycles were abolished. The pituitary gland of the treated animals was heavier than that of the control group, largely as a result of an increase in the volume of tissue occupied by chromophobe cells; the portion of the basophil cells was lower. The authors concluded that, in the rat, inhibition of fertility with compound XIX may be due to a variety of factors, including inhibition of ovulation, nidation, ova transport, and endometrial differentiation.

Bruce (1959) studied the effect on female mice, following oral administration of 0.25 mg and 0.5 mg per day for 32 days of 17α-ethynyl-19-nortestosterone. The treatment, 0.5 mg dose, produced inhibition of the estrous cycle, but not of mating. Upon cessation of the treatment, normal estrous cycles were restored, and ovulation, fertilization, and implantation occurred normally. It was suggested that the treatment may have delayed, rather than inhibited ovulation. The inhibition of estrous cycle in the rat, following subcutaneous administration of 0.5 mg/kg, was reported by Richter (1958).

Lerner *et al.* (1962) reported that 16α,17α-dihydroxyprogesterone acetophenone inhibited estrus in the rat following daily subcutaneous administration (1 mg/day) or administration in the diet (15 mg/day). Daily subcutaneous administration of 0.8 mg prevented mating in 100%; a dose of 0.16 mg/day was effective in only 70%.

TABLE XXXVII

ANTI-FERTILITY ACTIVITY OF 17α-ETHYNYLESTRADIOL-17β 3-METHYLETHER FOLLOWING ORAL ADMINISTRATION

Daily dose (μg)	Number of animals	Average number of implantation sites	% Inhibition
3.0	3	8.0	0
15.0	3	4.0	33
75.0	3	0.0	100

Falconi and Ercoli (1963) found that a daily dose of 17α-ethynylestradiol-17β 3-cyclopentylether, 5.46 μg, given orally for 30 days, fully inhibited the fertility and estrous cycle in the female rat (Table XXXVII). A similar dose of 17α-ethynylestradiol-17β 3-methylether was not effective.

TABLE XXXVIII

EFFECT OF VARIOUS STEROIDS IN INHIBITING IMPLANTATION IN RATS AND MICE[a] (SUBCUTANEOUS ADMINISTRATION)

Steroid	Dose level studied (mg)	Effective dose (mg) Rats	Mice
5α-Androstane-3,7,17-trione	0.5–2.5	Inactive	
3β-Acetacetoxy-3α-acetoxy-androstan-17-one	0.5–2.5	Inactive	
3α-Ethynyl-3β-hydroxy-A-nor-5α androstan-17-one	0.5–2.5	Inactive	
3α-Ethynyl-A-nor-5α-androstane-3β,17β-diol	0.02–2.5	0.5	0.5
3α-Ethynyl-A-nor-5α-androstane-3β,17β-diol diacetate	0.5–2.5	Inactive	
17α-Ethynylestra-3,5-diene-3,17β-diol diacetate	0.1–2.5	0.5–2.5	0.1
6α-Thioacetylpregn-4-ene-3,11,20-trione	0.5–2.5	Inactive	
17-Thiomethylestra-1,3,5(10),16-tetraen-3-ol	0.1–1.0	1.0	0.1
19-Acetoxyandrosta-1,4,diene-3,17-dione	0.1–1.0	1.0	0.1

[a] Data from Banik and Pincus (1962).

Banik and Pincus (1962) studied the effect of steroids, on implantation, when these materials were administered subcutaneously or orally to adult rats and mice. The test substances were given from the first day of pregnancy for 3 days. At autopsy, between day 10 and 12 after mating, the number of implanted fetuses was counted. Table XXXVIII summarizes the results following injection, and Table XXXIX deals with the results after oral administration. A similar test was used by Overbeek *et al.* (1962), who treated rats during one cycle (4 days) orally with the test substance; at the end of the treatment they inspected the oviduct for the presence of eggs. The authors reported that

TABLE XXXIX

EFFECT OF VARIOUS STEROIDS IN INHIBITING IMPLANTATION IN RATS[a]
(ORAL ADMINISTRATION)

Steroid	Dose level studied (mg)	Effective dose (mg)
3α-Ethynyl-*A*-nor-5α-androstane-3β,17β-diol	2.5–15.0	15.0
17α-Ethynylestra-3,5-diene-3,17β-diol acetate	0.5–15.0	15.0

[a] Data from Banik and Pincus (1962).

TABLE XL

ACTIVITY OF VARIOUS STEROIDS ADMINISTERED ORALLY IN INHIBITING OVULATION IN THE RAT[a]

Steroid	Daily dose (mg)	Rats with eggs in oviduct
None	0	7/8
17α-Ethynylestradiol-17β 3-methylether (MEE)	0.06	9/9
	0.12	6/7
	0.24	3/12
17α-Ethynyl-19-nortestosterone	4.0	6/6
17α-Ethynyl-19-nortestosterone + MEE (30 μg)[b]	2.0	5/6
	4.0	9/14
	8.0	8/18
17α-Ethynyl-17β-hydroxyestr-5(10)-en-3-one	2.0	13/26
	4.0	5/21
17α-Ethynylestr-4-en-17β-ol	2.0	12/18
	4.0	5/24
17α-Ethynylestr-4-en-17β-ol + MEE (30 μg)[b]	2.0	10/25
	4.0	3/21

[a] Data from Overbeek *et al.* (1962).
[b] Administered in two equally divided doses.

the activity of various compounds was enhanced by low doses of an estrogen. Progesterone, by injection, was effective at a dose of 1 mg (twice a day for 4 days) alone, and at a dose of 0.5 mg if 15 μg of ethynylestradiol 3-methylether was added. Table XL gives the activity of various steroids studied by oral administration.

During pregnancy, high doses of estrogens will produce abortion or fetal resorption in many animal species. Schofield (1962) reported that in the rabbit a daily dose of estradiol 17β-benzoate, 5 μg, given after mating for 3 days, produced infertility; if the injections were given on days 2, 3, and 4 after mating, a daily dose of 10 μg had to be used. It appears that this relatively low dose level, given early after mating, might have inhibited fertility by another mechanism, perhaps by influencing the ova transport in the oviducts.

VI. Activity of Steroids in Man

Clinical conditions in which it may be desirable to inhibit the production of gonadal hormones may include syndromes associated with hypersecretion, hormone-dependent cancer, and fertility control.

The prediction that population explosion will people the entire surface of the earth by 2026 A.D. (von Foerster *et al.*, 1960) is obviously an exaggeration of the possible projection, but it does emphasize the fact that if the present rate of population growth is not stemmed artificially, it will be slowed down by high death rates resulting from catastrophic unregulated growth. Of the great number of active steroids, which were described in the preceding section, only less than a dozen have been evaluated clinically, and the effect on the pituitary function and ovulation inhibition studied. Only three products are at present available commercially, 17α-ethynyl-19 nortestosterone (norethisterone), the 17-acetate, and the $\Delta^{5(10)}$ analog (norethynodrel); the recommended dosages vary from 2.5 to 10.0 mg per day, and all preparations contain varying amounts of 17α-ethynylestradiol-17β, or of the 3-methylether, from 50 to 150 μg.

A. Gonadotropin Inhibition

The activity of estrogens administered orally for short periods of time in decreasing gonadotropin output in castrated or menopausal women has been studied by Howard *et al.* (1956) and Rosemberg and Engel (1960). Both groups of investigators assayed the levels of urinary gonadotropin using uterine hypertrophy in immature mice as the end point. Conjugated estrogens (standardized as estrone sulfate) were

stated to be active in the 2.5–10.0 mg range per day. Equilin sulfate was active at 0.6–1.2 mg dose levels; a mixture of sulfonated urinary estrogens (Premarin[R]) had an intermediate activity and a dose of 1.2–2.5 mg per day was needed to suppress significantly urinary gonadotropin levels. Rosemberg and Engel (1960) found methyltestosterone to be ineffective in doses of 5–100 mg per day, when administered orally, and an effective dose of norethisterone was 20 mg per day for 5 days.

Tokuyama *et al.* (1954) studied 21 patients who were menopausal or castrated for effective gonadotropin suppression with oral estrogen. Stilbestrol was effective at the 1 mg dose, and in decreasing order of activity conjugated equine estrogens at 2 mg, potassium estrone sulfate at 4 mg, and estradiol-17β and potassium estradiol-17β sulfate at 5 mg.

Progesterone was reported to be an active anti-gonadotropin by Rothchild (1957); 100 mg, given by intravenous infusion was stated to decrease urinary gonadotropin values within 24–48 hours. Epstein *et al.* (1958) compared the effect of 17α-ethyl-19-nortestosterone, 17α-methyl-19-nortestosterone, and 17α-ethynyl-17β-hydroxyestr-5(10)-en-3-one, given intramuscularly to patients, male and female, associating hypogonadism with high follicle-stimulating hormone excretion; they found that the 17α-methyl compound was most effective on a weight basis (20–40 mg per week). 17α-Hydroxyprogesterone caproate, in doses up to 500 mg intramuscularly once a week, did not alter FSH excretion significantly.

The influence of testosterone propionate and methyltestosterone on the suppression of pituitary gonadotropins in castrated men was reported by Catchpole *et al.* (1942). After 10–13 days of intramuscular injections with 20 mg of testosterone propionate per day, the gonadotropins were significantly decreased in the urine and remained down even after 3 months of therapy; the gonadotropin titers returned to the pretreatment levels 7–13 days after cessation of treatment. Methyltestosterone, orally at 70 mg per day, was effective.

The effect of testosterone propionate and 2α-methyl-17β-propionoxy 5α-androsten-3-one on the urinary excretion of pituitary gonadotropin was studied by Blackburn and Albert (1959). Post-menopausal patients with cancer of the breast received the steroids by intramuscular injection, 100 mg three times weekly. The propionate of 2α-methyl dihydrotestosterone (VI) was not effective under these conditions whereas testosterone propionate therapy resulted in decreased urinary gonadotropin values. Indirect evidence on the suppression of pituitary activity with 19-nortestosterone derivatives has been obtained by Ferin and collaborators. 19-Nortestosterone cyclopentyl propionate given by intramuscular injection, 100 mg per dose (300–900 mg total amount)

produced postponement of menstruation in some cases and atrophic endometrium (Ferin, 1955); 2.5–5.0 mg of 17α-methyl-19-nortestosterone (XVIII) or a 3-deoxy compound, 17α-ethynylestr-4-en-17β-ol (XXXIII) produced a state of prolonged amenorrhea with sterility, lasting up to 30 months. The compounds were administered sublingually to normally menstruating women (Ferin, 1962). It has been suggested that compound XVIII may work either by inhibiting the pituitary function or by exerting anti-estrogenic activity (Ferin *et al.*, 1960).

Tyler and Olson (1958) used norethisterone, norethynodrel, and 17α-hydroxyprogesterone caproate for their "rebound effects" in ovulation with good success. The first two compounds were given at a dose of 10 mg, orally, for about 10 days and the third compound by intramuscular injection, 1½ ml followed a week later by another 1 ml (125 mg) injection.

In short term therapy (10–16 days), no depression in urinary gonadotropin excretion, following sublingual therapy with 17α-methyl-5α-androstane-3α,17β-diol in doses of 50 and 200 mg/day, or for 400 mg/day of 17α-methylandrost-5-ene-3β,17β-diol (Clayton and Prunty, 1958). Testosterone propionate, given by intramuscular injection, was found active at the dose of 100 mg, three times per week. 17α-Ethyl-19-nortestosterone, given orally, was ineffective at a dose of 100–150 mg/day, but a dose of 200 mg produced depression of urinary gonadotropin values (Clayton and Prunty, 1958). Norethisterone enanthate, 100 mg weekly for 6 weeks, was found active by Martin and Cuningham (1960) in patients with metastic mammary carcinoma. Feldman (1960) found 17α-methyl-19-nortestosterone to be active at a dose of 30 mg t.i.d. This regimen produced fluid retention also, and moderate virilization. Segaloff *et al.* (1959) found 19-nortestosterone propionate ineffective as an anti-gonadotropin in breast cancer patients.

B. Inhibition of Fertility

Rock *et al.* (1956) studied the effect of norethisterone (17α-ethynyl-19-nortestosterone, XX), the $\Delta^{5(10)}$ analog norethynodrel (17α-ethynyl-17β-hydroxyestr-5(10)-en-3-one, XIX), and norethandrolone (17α-ethyl-19-nortestosterone, XVIII) in normal, healthy women at ages ranging from 22 to 39 years. The compounds were given by mouth in dosages of 5–50 mg per day from days 5–25 of the menstrual cycle. These studies revealed that all three compounds were potent suppressors of ovulation as demonstrated by a consistent reduction in pregnanediol excretion in the second half of the menstrual cycle and a

tendency for a decrease in 17-keto steroid output. Early menstrual bleeding occurred only in a few subjects, especially in those who received low dose of norethisterone and norethynodrel, but with more frequency in subjects who were given norethandrolone even at dosages of 10–50 mg. In a later study, Pincus *et al.* (1958) reported on the effect of these compounds, with added amounts of an estrogen, 17α-ethynylestradiol-17β 3-methylether (XVI), 0.1–0.22 mg. Basal temperature curves, 17-keto steroid and pregnandiol excretion were measured, and endometrial biopsies and vaginal smears were taken. Compounds XX and XIX were given in dosages of 10 and 20 mg, and XVII in dosages

TABLE XLI

INHIBITION OF OVULATION WITH ORALLY ADMINISTERED STEROIDS IN THE NORMAL FEMALE[a]

	Indices of ovulation		
Compound	Basal temperature rise	Pregnandiol (mg/day)	17-Keto steroid (mg/day)
None	53/56	2.06	6.98
Norethisterone XX (all sites)	1/28	0.33	7.16
XX+XVI	0/3	0.37	4.93
Norethynodrel XIX (all doses)	0/36	0.31	5.88
XIX+XVI	5/102	0.29	5.66
XVII (all doses)	1/17	0.34	4.50
XVII+XVI	2/8	0.44	5.03

[a] Data from Pincus *et al.* (1958).

of 10–50 mg. The authors stated that ovulation was inhibited just as well with the pure compounds as by the compounds with added estrogen, on the basis of an absence of the rise in basal temperature, decreased urinary pregnandiol, characteristic postovulatory endometrium, and the ovulation time change in the vaginal cytology. Slight, but significant, decreases in urinary 17-keto steroids were observed (Table XLI). Added estrogen, however, decreased the incidence of breakthrough bleeding. Other groups were able to confirm inhibition of ovulation following oral therapy with 19-nor steroids. These include Goldzieher *et al.* (1962) and Rice-Wray *et al.* (1962), who used a preparation consisting of 10 mg of 17α-ethynyl-19-nortestosterone (XX) and 0.06 mg of 17α-ethynylestradiol-17β 3-methylether

(XVI) Other combinations of orally active progestational compounds and estrogens reported as active, are given in Table XLII.

A new therapeutic method, sequential therapy, has been suggested by Goldzieher *et al.* (1963), who used 80 μg of 17α-ethynylestradiol-17β 3-methylether for 15 days beginning on the 5th day of the menstrual cycle, followed by 5 days of a combination of the same estrogen with 2 mg per day of a progestogen, 6-chloro-17α-acetoxypregna-4,6-diene-3,20-dione. Urinary pregnandiol levels of patients so treated were found to be low, indicating the absence of ovulation.

TABLE XLII

ORALLY ACTIVE STEROIDS; INHIBITION OF FERTILITY IN WOMEN[a]

Progestogen (daily dose, mg)	Estrogen (daily dose, mg)
Norethynodrel (5.0)	MEE[b] (0.075)
Norethynodrel (2.5)	MEE (0.1)
Norethisterone acetate	MEE (0.05)

[a] Data from Mears (1962)

[b] MEE—17α-ethynylestradiol-17β 3-methylether.

The effect of various progestational agents on the ovaries and pituitaries of female baboons (*Papio hamadrya*) has been investigated. The compounds were given orally; the dose and duration was as indicated. At the end of the experiment, the ovaries and pituitaries were examined histologically. 17α-Acetoxyprogesterone 3-cyclopentyl ether did not interfere with pituitary morphology, whereas treatment with 6α-methyl-17α-acetoxyprogesterone produced histological changes (degranulation of the basophils) of the pituitary. The ovaries of treated monkeys were atrophied and contained only cystic follicles, but no corpora lutea (Cavallero *et al.*, 1959; Goisis *et al.*, 1960; Goisis and Mosca, 1962).

Appendix—Steroid List

Roman numeral reference	Compound
I	Testosterone
II	17β-Hydroxy-5α-androstan-3-one
III	Androst-4-ene-3,17-dione
IV	3α-Hydroxy-5α-androstan-17-one (androsterone)
V	2α-Hydroxymethyl-17β-hydroxy-5α-androstan-3-one
VI	2α-Methyl-17β-hydroxy-5α-androstan-3-one
VII	6α-Fluoro-17β-hydroxyandrost-4-en-3-one
VIII	6α-Chloro-17β-androst-4-en-3-one acetoxy
IX	6α,17α-Dimethyl-17β-hydroxy-5α-androstan-3-one
X	2-Cyano-5α androst-2-en-17β-ol acetate
XI	2-Formyl-5α-androst-2-en-17β-ol
XII	2α-Formyl 5α-androstan-17β-ol
XIII	2-Hydroxymethylene-17α-methyl-17β-hydroxy-5α-androstan-3-one
XIV	17α-Methyl-17β-hydroxyandrosta-1,4-dien-3-one
XV	17α-Ethylestr-4-en-17β-ol
XVI	17α-Ethynylestradiol-17β 3-methylether
XVII	17α-Ethyl-17β-hydroxyestr-4-en-3-one
XVIII	17α-Methyl-17β hydroxyestr-4-en-3-one
XIX	17α-Ethynyl-17β-hydroxyestr 5(10)-en-3-one
XX	17α-Ethynyl-17β-hydroxyestr-4-en-3-one
XXI	Pregn-4-ene-3,20-dione
XXII	6α-Methyl-17α-acetoxypregn-4-ene-3,20 dione
XXIII	19-Norprogesterone
XXIV	6-Chloro-17α-acetoxypregna-4,6-diene-3,20-dione
XXV	17α-Acetoxy-19-norpregn-4-ene-3,20-dione
XXVI	6,16α-Dimethylpregna-4,6-diene-3,20-dione
XXVII	6β,16α-Dimethylprogesterone
XXVIII	3β-Fluoro-17α-acetoxypregn-5-en-2-one
XXIX	17α Acetoxypregn-5-en-20-one
XXX	17α-Acetoxypregna-3,5-dien-20-one
XXXI	6-Dehydro-retroprogesterone
XXXII	17α-Ethylestr-4-en-17β-ol
XXXIII	17α-Ethynylestr-4-en 17β-ol
XXXIV	3-Methoxy-16α-methylestra-1,3,5(10)-triene-16β,17β-diol

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Chapter 4

The Treatment of Human Malignancies with Steroids

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Our knowledge of hormonal therapy of malignant lesions in man is still in its infancy. It has long been established that castration or the administration of estrogens produces striking clinical remissions, occasionally accompanied by objective regression, of human prostatic cancer. I am not aware of any studies which can be used as a basis for comparison of the relative efficacy of the various estrogens when used as primary hormonal therapy of prostatic cancer. Indeed, there is evidence that secondary hormonal therapy is of no benefit after failure or escape from the good effects of either castration or estrogen administration (Brendler and Prout, 1962; Brendler, 1965).

Recently it has become evident that in a reasonable percentage (circa 25–30%) of patients with metastatic carcinoma originating in the endometrium of the uterus, objective regressions are obtained through administration of adequate amounts of progestational hormones (Kelley and Baker, 1960). A combination of surgical and roentgen therapy in this uncommon tumor results in a high cure rate, thus, fortunately, leaving few patients with recurrent disease available for study (Hertig and Gore, 1960).

The acute leukemias of children are often exquisitely sensitive to active adrenal cortical hormones, striking and sometimes long-lasting remissions being achieved. However, again I am not aware of any attempts to show that this property is displayed to a greater or lesser extent by one or another type of corticoid, nor of any well documented, concerted effort to find corticoids which would induce remissions either in higher percentage or of longer duration.

Breast cancer, to my knowledge, is the only malignant disease for which a concerted effort has been made to delineate the hormonal factors in continued tumor growth and to utilize this to improve the favorable results of hormonal therapy. Fortunately for our purposes, cancer of the breast is common enough in man and animals to supply material for study, and ample supplies of a suitable reference material in man, testosterone propionate, are available so that comparisons can be made.

As a biologist, I am dismayed at the small size of the clinical samples upon which we must base our conclusions. On the other hand, as a

clinician, I am proud of our clinical colleagues who have labored so hard and so long to provide an ever-increasing body of information and, we hope, ever-improving results.

Cancer of the breast does not follow the classical pattern of inexorable growth with measured cadence, but shows an amazingly variable growth pattern with instances of extremely rapid growth and spread, as well as slow growth or apparent dormancy for many years and then sudden, rapid growth. Between these two extremes are tumors of both steady and intermittent growth and spread. It is important to be aware of the natural history of the disease and to make an effort to confine our palliative therapies such as hormones to truly advancing cases, so that patients will not be given an effective therapy when the disease is dormant which might then deprive them of a potentially good response when the tumor subsequently again springs to life and growth.

It is generally agreed that in women who are still menstruating or are only recently (less than one year) postmenopausal, castration is the therapy of choice. This induces a significant number of remissions which frequently last a long time and do not prejudice subsequent response to administrative or ablative hormonal therapy. Indeed, there is evidence that administrative hormonal therapy is often more effective after than prior to castration. In other words, "medical castration" by means of testosterone or a similar agent is not as effective as surgical or roentgen ray castration, which still permits a good objective response to the subsequent administration of testosterone and its related agents (Gordon and Segaloff, 1958).

An increasing mass of carefully collected and analyzed information on the response of breast cancer to testosterone propionate is becoming available. Much of this is from the large number of studies carried out by the Cooperative Breast Cancer Group (1961, 1962) of the Cancer Chemotherapy National Service Center. All the Group's data have been collected, using a careful protocol with good statistical methodology. Each case is reviewed by two members of the Group from other institutions in order to assess the evaluation as well as to obtain conformity to the criteria set forth in the Group's protocol. This gives the data a high order of reliability and reproducibility. Although the Group does not claim that their method of evaluation and decision as to remission rate is the only one or even the best, it is reproducible and measurable.

What have they found in their patients treated with testosterone propionate? The question most frequently asked is whether or not such arbitrarily defined objective remissions do increase survival. The answer can now be given and fortunately is in the affirmative (Coopera-

tive Breast Cancer Group, 1962). The objective regressions are indeed accompanied by increased longevity. In fact, the period of remission is not only one of substantial subjective improvement, frequently with return of ability to carry on usual tasks, but also one that may be termed "a vacation from death." The testosterone reference data of the Cooperative Breast Cancer Group have also proved several other items, many of which were previously speculative.

First, the objective remission rate is significantly increased with increasing menopausal age. This slope is greatest for disease confined to local recurrences in soft tissue and least for disease mainly involving the skeletal system. In this area it was first necessary to make the judgment based on previous work by members of the Group, especially the analysis of the data of the Sloan-Kettering Institute by Escher (1958) that patients whose major site of metastatic disease is visceral, including lung and central nervous system, have the worst prognosis for response and longevity; that patients with local recurrence without significant involvement of the viscera or the skeletal system have the best prognosis for longevity; and that patients whose metastatic disease is mainly in the skeleton have an intermediate prognosis. These judgments proved to be correct, as the testosterone propionate control data have borne out the judgments

These data indicated an overall response rate of approximately 20% or a little better. The variation in response rate between the different groups has defined the problem of precisely evaluating agents. We must ignore in the literature heterogeneous preselected or postselected samples for analysis. We must have the data in a form so that results may be compared with those of a reference material We must be careful, if a given study contains many patients with a poor prognosis (such as the less-than-one-year post-castration patient with visceral disease), or the high objective response rate group of locally recurrent disease in patients more than 10 years postmenopausal, to take such factors into account. Obviously, for evaluations, samples of less than 20 patients each, on both experimental compound and standard, represent an inordinate risk. Even samples of this size represent a substantial risk of missing good compounds, but they are at least manageable in terms of accessibility of materials, patients, and completion of observations within a reasonable length of time. Such a design permits, with about a ±5% error, the rejection of compounds as less active than testosterone propionate. It does not permit an estimate of relative activity other than this. Such a judgment requires using more patients on both the experimental and control materials. Therefore, the studies which essentially fit these criteria and those being published by the Cooperative Breast

Cancer Group and others (European Breast Cancer Group, 1963), that is, adequate criteria, randomization, and outside review, will form the basis for the conclusions to be drawn in this report.

The problem of dose response curves must be ignored in this present approach, since it is necessary to keep the physiologic effects (frequently misnamed "side effects") of potent hormonal agents within reasonable bounds for the patient and still give a sufficient amount of the agent to maintain the objective remission rate. In some studies employing smaller doses of androgen, such as 25 or 50 mg of testosterone propionate 3 times a week, remission rates have been distinctly less; while those studies using 200 mg or more of testosterone propionate 3 times a week have had a very high incidence of intolerable virilization. Therefore, 100 mg of testosterone propionate 3 times a week seemed the most reasonable dosage, and the Cooperative Breast Cancer Group and others working in this area have generally attempted to equate either androgenicity or total dose of steroid to this level. The same has been done with estrogens to a lesser degree, where we equated to 15 mg equivalent of diethylstilbestrol daily.

Early in our own studies, we had hoped that we could readily find a testosterone metabolite or some common denominator in hormonal pattern which could be exploited and rapidly improve our percentage of regressions in this dread disease. Unfortunately, the task to date has not proved that simple. We have learned a great deal, we have abandoned many of our hypotheses in the process, we have developed new ones, and we hope that we have improved the care and prognosis of the average patient with advancing breast cancer and that we will do this at an ever-increasing pace in the future.

The two earliest examples of hormonal agents effective against cancer of the breast are testosterone propionate and diethylstilbestrol, a strong androgen and a potent estrogen. At first, these two hormones, representing major androgenic and major estrogenic activities, appear totally dissimilar, but they share one property in common, namely, that they are able to produce substantial decrease in the urinary excretion of gonad-stimulating hormone in the dosages mentioned. As we study them even more thoroughly this seems to be the major area of hormonal similarity. Thus, when our earliest attempts to find improved compounds led to the testing of intermediates in the chemical synthesis of testosterone and some other androgens of lesser potency, and these neither proved to be clinically effective against advancing breast cancer nor to have the ability of decreasing urinary gonad-stimulating hormone, we were led to the thesis that there seemed to be a correlation between ability to lower gonad-stimulating hormones in the urine and clinical

effectiveness. This view was further strengthened when we found that methyltestosterone, another strong androgen, shared both the clinical effectiveness and the gonad-stimulating hormone lowering properties (Segaloff *et al.*, 1953).

These findings have stimulated us and others to look for compounds particularly effective in lowering gonad-stimulating hormone, hopefully without the pronounced sexogenic effects of the potent androgens and estrogens.

This may indeed still represent a useful search, since we know of no compound that will substantially lower the gonad-stimulating hormone in the urine which is not also clinically effective against advancing breast cancer. However, this is obviously not a prerequisite for optimal antitumor activity, since we already have at least three compounds which do not differ significantly from our reference standard, testosterone propionate, in their clinical effectiveness, yet which have little or no potency in lowering the urinary titer of gonad-stimulating hormone. These compounds are: 2α-methyldihydrotestosterone propionate (Blackburn and Childs, 1959), fluoxymesterone (Segaloff *et al.*, 1958), and Δ^1-testololactone (Segaloff *et al.*, 1960). Thus we have here a finding to which as yet we have no exceptions, that compounds which strongly lower gonad-stimulating hormone in the urine are clinically effective against breast cancer. However, there are hormonal agents that lack this property but still have clinical effectiveness against cancer of the breast.

Androgenicity poses a similar problem. Among the androgens examined, it originally appeared that any change which decreased the androgenic potency of the compound was accompanied by a decrease in clinical effectiveness. However, there are now androgenic hormones of significantly less androgenicity than testosterone which still retain clinical effectiveness. The best known one in this area is 2α-methyldihydrotestosterone propionate (Cooperative Breast Cancer Group, 1961), which is as effective clinically as testosterone propionate but is significantly less androgenic, as found in double blind studies. Nevertheless, this does not gainsay the fact that this is still a substantially androgenic agent.

On the other hand, I know of no androgens that are as androgenic as testosterone propionate in man which do not have substantially the same clinical effectiveness. This is the major reason why I have felt that it is important to test androgens more potent than testosterone in hopes that they will have markedly increased clinical efficacy. Until very recently this had not been possible, since there are no androgens which had previously been known to be substantially more androgenic than

testosterone propionate in man. The group at Upjohn and we ourselves have recently reported extensively on the chemistry and biology of a group of 19-nor-7α-methyltestosterone derivatives (Campbell *et al.*, 1963; Lyster and Duncan, 1963; Segaloff, 1963). As yet it is too early to evaluate the clinical effectiveness of any of these agents, but we do have preliminary evidence that one of these compounds is considerably more androgenic than testosterone propionate in man, and we hope that it will have greater clinical efficacy also.

One of the major problems that is brought up by the question of androgenicity in man is our very real difficulty in equating androgenicity, except for substantially similar compounds in the same dose given in a double blind fashion. We treat a group of women rather heterogeneous with regard to voice tone, general body hair distribution, musculature, and subjective responses to medication. In addition to this, they are all patients who are seriously ill with advancing cancer, who may or may not go to great lengths to conceal or flaunt the objective and subjective evidences of androgenicity. Thus it must be recognized that the problem of assessing androgenicity is a function of both the patient and the physician. For this reason, the best data are derived from double blind comparisons. One must then add to this the variation in duration of treatment. There have been innumerable unsuccessful attempts to evaluate androgenicity adequately, such as attempts to measure changes in voice quality, rate of hair growth, and musculature, etc., yet we still must make our evaluation on clinical grounds, trying to classify as many patients as possible on a given agent and to decide whether it is more or less virilizing than our reference standard.

Next we should consider the assessment of metabolites of compounds known to be effective against advanced breast cancer. Here it is entirely possible that we are studying the wrong compounds, but a great many of the obvious estrogen, androgen, progestational, and corticoid metabolites have been tried clinically without finding any compounds more effective clinically than their precursors. We have learned many interesting facts from these studies. For example, in man as in animals, the saturation of the 4,5 double bond in testosterone leads to a compound, 5α-dihydrotestosterone (Segaloff *et al.*, 1955) which is indistinguishable from testosterone propionate in its clinical effectiveness and androgenicity. On the other hand, saturation of the same double bond with the 5β configuration, producing 5β-dihydrotestosterone, one of the etiocholanolones, did not prove to be clinically effective or androgenic, but it did produce fever in the patients so treated, giving us our first instance of so-called "etiocholanolone fever" (Segaloff *et al.*, 1957). Many of the known and potential monohydroxylated metabolites

of testosterone—the 11β-hydroxy, 6β-hydroxy, 2α-hydroxy, 16α-hydroxy testosterones—have been tested, but none has been as effective as the parent compound (Cooperative Breast Cancer Group, 1961; Goldenberg and Hayes, 1961).

We do not know what will ultimately prove to be the complete set of intermediate metabolites of any of the effective steroids. The number of metabolites of the natural estrogens seems to be increasing rapidly of late, but we hope that it will be possible to test all proven metabolites and that perhaps one or more of them will prove to be clinically effective.

Because of the apparent correlation for estrogens and androgens between potency and clinical activity, an effort has been made to find and test compounds of particular potency in each of the spheres of hormonal activity. Some of these have been completed, others are under way. At present, there is no evidence that progestational agents, even some extremely potent ones such as 6α-fluoro-17-acetoxyprogesterone and 6α-methyl-17-acetoxyprogesterone, or parenteral agents such as the 17-caproate of 17-hydroxyprogesterone have proved to be effective.

Much has been written about the role of adrenal cortical hormones in the treatment of advanced breast cancer. This area still requires investigation and is being actively explored. So far, the studies sponsored by the Cooperative Breast Cancer Group (1961) and others (Gardner *et al.*, 1962; Dao *et al.*, 1961) indicate that the usual corticoids are effective against advancing cancer of the breast, but that much of the improvement is subjective, and objective regressions occur less frequently than with testosterone propionate. There are, however, a few notable exceptions, and we hope that there will soon be more. The animal antitumor studies reported by Glenn and associates (1960) found a multisubstituted 21-deoxy corticoid to be the most effective agent in two tumor systems, the C_3H mouse spontaneous mammary adenocarcinoma and the testosterone-resistant strain of the Huggins rat fibroadenoma. This compound, oxylone acetate, as well as its free form, oxylone, is a potent corticoid in man and does not differ significantly from testosterone propionate in its ability to produce objective regression of advanced mammary carcinoma in the studies performed by the Cooperative Breast Cancer Group to date (1961) and by Kelly and Talley (1960). Other 21-deoxy corticoids are being studied in animal systems, and a few are currently under preliminary study in man. We hope that this will be an exploitable lead to corticoids particularly effective against breast cancer.

There is conflicting evidence that replacement dosage of hydro-

cortisone or cortisone, with or without triiodothyronine or thyroid, may be responsible for the objective regressions seen with adrenalectomy and hypophysectomy, but large numbers of objective regressions (Gardner *et al.*, 1962) have been reported with a low dose corticoid-triiodothyronine combination. These combinations will naturally require more diligent studies, since there are also reports that replacement corticoid is not effective (Dao *et al.*, 1961).

It has also seemed reasonable to test compounds which display one or more hormonal properties to an unusual degree in relation to their other hormonal properties. The most obvious of these are those in which anabolic activity predominates over androgenic activity, or salt retention predominates over corticoid manifestations. To date I am not aware of any compound of this type which is highly effective in cancer of the breast.

There are no animal tumor systems which seem to have a high degree of correlation with this developing breadth of clinical knowledge. However, it does seem important to exploit all possible leads, and since many of these compounds are particularly effective either in hormonally sensitive tumors or in standard tumor screens, compounds have been selected because they are outstanding in one or another of these areas. The best examples of compounds in this area are 2α-methyldihydrotestosterone propionate and oxylone acetate.

Finally, some compounds should be tested either because they are chemically unique despite absence of any of the other properties to which we have referred, or simply because they have not been tested. Since we have no better leads, I feel very strongly that we must continue to test some compounds in this area.

I have left to the last the assessment of compounds because of their ability to antagonize the hormonal properties of the body's natural hormones. This is an area which I consider full of promise, but one in which it is either difficult or impossible to be sure of what we are doing from hormonal assays. Also, we have not been able to find adequate numbers of compounds for testing, because either they are not available or we do not know how to find them—unless, of course, one is willing to consider an androgen as an estrogen antagonist and an estrogen as an androgen antagonist.

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Chapter 5

Comparative Objective Regressions Resulting from Steroid Treatment in Women Suffering from Breast Cancer

ALBERT SEGALOFF

The results of steroid treatment in women suffering from breast cancer are presented in two tables. Table I shows the published results that are available from the Groups using the procotol of the Cooperative Breast Cancer Group (1961, 1964). Table II consists of the author's studies prior to the availability of the Breast Group protocol. The patients of these studies were not selected in a random fashion. The subjects were usually admitted to the study on a consecutive basis. The results of these studies are listed as total objective regressions and total number of patients. Table II also includes some selected additional studies, including patients who do not meet the criteria of the protocol studies listed in Table I.

TABLE I

COOPERATIVE BREAST CANCER GROUP PROTOCOL STUDIES, 1956–1963

Steroid and dosage	Study	Dominant lesion	Objective regression/Number of patients: Menopausal age < 1 yr	1–5 yr	5–10 yr	10+ yr	Total
Testosterone propionate, 100 mg 3 × wk i.m.	Combined results (Cooperative Breast Cancer Group, 1961)	Breast	2/19	8/26	7/15	28/71	45/131
		Osseous	5/40	8/36	9/37	16/76	38/189
		Visceral	1/34	5/43	4/28	23/95	33/200
		Total	8/93	21/105	20/80	67/242	116/520
Testosterone propionate, 100 mg 3 × wk i.m	Gordan *et al.* (1963)	Breast	0/2	1/1	0/1	1/2	2/6
		Osseous	0/1	0/2	0/1	1/3	1/7
		Visceral	0/1	0/2	0/2	3/6	3/11
		Total	0/4	1/5	0/4	5/11	6/24
Fluoxymesterone (9α-fluoro-11β-hydroxy-17-methyltestosterone), 20 mg/day p.o.		Breast	0/1	0/1	0/1	0/1	0/4
		Osseous	0/1	0/1	1/1	1/4	2/7
		Visceral	0/0	1/2	0/3	1/5	2/10
		Total	0/2	1/4	1/5	2/10	4/21
Fluoxymesterone (9α-fluoro-11β-hydroxy-17-methyltestosterone), 20 mg/day p.o.	Combined results (Cooperative Breast Cancer Group, 1961)	Breast	0/3	1/4	0/4	4/13	5/24
		Osseous	0/8	0/9	1/5	4/22	5/44
		Visceral	0/8	1/9	3/8	2/13	6/38
		Total	0/19	2/22	4/17	10/48	16/106

Testosterone propionate, 100 mg $3 \times$ wk i.m.	Lewison *et al.* (1960)	Breast	0/0	2/2	0/0	4/6	6/8
		Osseous	0/1	1/2	1/1	0/2	2/6
		Visceral	0/2	0/2	0/1	0/3	0/8
		Total	0/3	3/6	1/2	4/11	8/22
9α-Fluoro-11-keto-17α-methyltestos terone, 40 mg/day p.o.		Breast	0/1	0/0	0/0	1/5	1/6
		Osseous	1/2	0/0	0/1	0/3	1/6
		Visceral	1/1	0/2	1/2	0/3	2/8
		Total	2/4	0/2	1/3	1/11	4/20
Fluoxymesterone (9α-fluoro-11β-hydroxy-17-methyltestosterone). 20 mg/day p.o.	Ansfield and Olson (1962). Cited in Ansfield *et al.* (1962)	Breast	0/0	0/0	0/0	2/4	2/4
		Osseous	0/0	0/2	0/1	1/6	1/9
		Visceral	0/1	0/2	1/1	1/5	2/9
		Total	0/1	0/4	1/2	4/15	5/22
17β-Hydroxy-17α-methyl-5α-androstane-3,11-dione, 100 mg/day p.o.		Breast	0/1	0/1	0/0	0/1	0/3
		Osseous	0/2	0/3	0/1	1/3	1/9
		Visceral	0/4	2/3	0/3	1/6	3/16
		Total	0/7	2/7	0/4	2/10	4/28
Testosterone propionate, 100 mg $3 \times$ wk i.m.	Carter *et al.* (1960)	Breast	0/1	0/0	0/0	0/2	0/3
		Osseous	1/2	0/1	0/1	2/4	3/8
		Visceral	0/2	0/2	0/1	2/6	2/11
		Total	1/5	0/3	0/2	4/12	5/22
19-Nor-17α-methyltestosterone, 40 mg/day p.o.		Breast	0/1	0/1	1/1	3/7	4/10
		Osseous	0/1	1/1	0/1	0/3	1/6
		Visceral	0/1	0/1	0/1	0/2	0/5
		Total	0/3	1/3	1/3	3/12	5/21

continued

TABLE I—*continued*

Steroid and dosage	Study	Dominant lesion	Objective regression/Number of patients: Menopausal age < 1 yr	1–5 yr	5–10 yr	10+ yr	Total
Testosterone propionate, 100 mg 3/wk i.m.	Hall and Haines (Cooperative Breast Cancer Group, 1961)	Breast	0/1	0/3	1/1	1/6	2/11
		Osseous	0/3	0/2	1/2	1/2	2/9
		Visceral	0/1	1/3	0/2	0/4	1/10
		Total	0/5	1/8	2/5	2/12	5/30
6-Dehydro-17α-methyltestosterone, 300 mg/wk p.o		Breast	0/1	0/2	0/0	0/4	0/7
		Osseous	0/3	0/2	0/1	0/2	0/8
		Visceral	0/1	0/1	0/1	1/2	1/5
		Total	0/5	0/5	0/2	1/8	1/20
Fluoxymesterone (9α-fluoro-11β-hydroxy-17 methyltestosterone), 10 mg/day p.o.	Volk *et al* (1962)	Breast	0/1	0/0	0/0	1/5	1/6
		Osseous	0/3	0/1	0/1	0/4	0/9
		Visceral	0/3	0/3	0/1	1/3	1/10
		Total	0/7	0/4	0/2	2/12	2/25
11β-Hydroxy-17α-methyltestosterone, 200 mg/day p.o.		Breast	0/1	0/0	0/0	1/6	1/7
		Osseous	0/2	1/2	0/0	0/4	1/8
		Visceral	0/2	0/3	0/0	0/3	0/8
		Total	0/5	1/5	0/0	1/13	2/23
Fluoxymesterone (9α-fluoro 11β-hydroxy-17-methyltestosterone), 20 mg/day p.o.	Dao (Cooperative Breast Cancer Group, 1961)	Breast	0/0	1/3	0/2	1/4	2/9
		Osseous	0/2	0/2	0/0	2/3	2/7
		Visceral	0/2	0/0	0/0	1/4	1/6
		Total	0/4	1/5	0/2	4/11	5/22

17α-Methyl-5α-androstane-$3\beta,11\beta,17\beta$-triol, 40 mg/day p.o.		Breast	0/1	0/3	0/2	0/2	0/8
		Osseous	0/1	0/3	0/1	0/3	0/8
		Visceral	0/2	0/1	0/1	0/4	0/8
		Total	0/4	0/7	0/4	0/9	0/24
Fluoxymesterone (9α-fluoro-11β-hydroxy-17-methyltestosterone), 20 mg/day p.o.	Talley *et al.* (1963)	Breast	0/1	0/1	0/1	1/2	1/5
		Osseous	0/3	1/3	0/3	0/3	1/12
		Visceral	0/0	0/1	2/3	1/4	3/8
		Total	0/4	1/5	2/7	2/9	5/25
$2\alpha,17\alpha$-Dimethyltestosterone, 50 mg/day p.o.		Breast	0/1	0/1	0/1	0/1	0/4
		Osseous	0/4	1/3	0/3	0/2	1/12
		Visceral	0/1	1/1	1/4	0/4	2/10
		Total	0/6	2/5	1/8	0/7	3/26
Testosterone propionate, 100 mg 3 × wk i.m.	Segaloff (1963)	Breast	0/0	0/1	0/0	0/1	0/2
		Osseous	0/0	0/1	0/0	0/1	0/2
		Visceral	0/2	0/2	0/1	1/2	1/7
		Total	0/2	0/4	0/1	1/4	1/11
17β-Hydroxy-17α-methyl-5β-androstan-3-one, 200 mg/day p.o.		Breast	0/1	0/2	0/0	0/3	0/6
		Osseous	0/1	0/0	0/0	0/1	0/2
		Visceral	0/5	0/1	0/2	0/5	0/13
		Total	0/7	0/3	0/2	0/9	0/21
Testosterone propionate, 100 mg 3 × wk i.m.	Blackburn (1959)	Breast	0/0	0/2	0/1	0/0	0/3
		Osseous	0/1	1/3	0/2	1/5	2/11
		Visceral	0/1	1/3	0/1	0/2	1/7
		Total	0/2	2/8	0/4	1/7	3/21

continued

TABLE I—*continued*

Steroid and dosage	Study	Dominant lesion	Objective regression/Number of patients				
			Menopausal age				
			< 1 yr	1–5 yr	5–10 yr	10+ yr	Total
2α-Methyl-dihydrotestosterone propionate (17β-hydroxy-2α-methyl-5α-androstan-3-one propionate), 300 mg/wk i.m.		Breast	0/1	0/2	0/2	1/1	1/6
		Osseous	1/1	3/3	2/2	2/6	8/12
		Visceral	0/2	1/3	1/2	1/2	3/9
		Total	1/4	4/8	3/6	4/9	12/27
2α-Methyldihydrotestosterone propionate (17β-hydroxy-2α-methyl-5α-androstan-3-one propionate), 300 mg/wk i.m.	Goldenberg, Gordan, and Blackburn (Cooperative Breast Cancer Group, 1961)	Breast	0/3	0/4	0/4	5/9	5/20
		Osseous	1/7	3/8	6/10	4/12	14/37
		Visceral	0/7	2/11	2/7	2/14	6/39
		Total	1/17	5/23	8/21	11/35	25/96
Testosterone propionate, 100 mg 3 × wk i.m.	Blackburn (Cooperative Breast Cancer Group, 1961)	Breast	0/1	0/1	0/1	0/0	0/3
		Osseous	0/1	1/1	2/2	0/2	3/6
		Visceral	0/1	0/3	0/1	3/6	3/11
		Total	0/3	1/5	2/4	3/8	6/20
2α-Methyldihydrotestosterone (17β-hydroxy-2α-methyl-5α-androstan-3-one), 200 mg/day p.o.		Breast	0/1	0/0	0/0	0/1	0/2
		Osseous	0/2	1/1	1/3	1/2	3/8
		Visceral	0/2	0/2	2/2	2/5	4/11
		Total	0/5	1/3	3/5	3/8	7/21

Androstanediol dipropionate (5α-androstane-$3\alpha,17\beta$-diol dipropionate), 100 mg 3 × wk i.m.	Segaloff *et al.* (1961b)	Breast	0/1	0/0	0/1	0/2	0/4
		Osseous	0/2	0/2	0/1	0/4	0/9
		Visceral	0/2	2/2	0/1	0/5	2/10
		Total	0/5	2/4	0/3	0/11	2/23
Androsterone, 100 mg 3 × wk i.m.	Segaloff *et al.* (1961a)	Breast	0/2	0/2	0/1	0/4	0/9
		Osseous	0/1	0/1	0/1	0/3	0/6
		Visceral	0/1	0/1	0/0	1/5	1/7
		Total	0/4	0/4	0/2	1/12	1/22
Testosterone propionate, 100 mg 3 × wk i.m.	Brennan *et al.* (1960)	Breast	0/1	0/1	0/1	1/3	1/6
		Osseous	0/5	0/2	0/1	0/3	0/11
		Visceral	0/0	0/2	0/1	0/3	0/6
		Total	0/6	0/5	0/3	1/9	1/23
5α-Androstane-$3\beta,17\beta$-diol, diacetate, 300 mg/wk i.m.		Breast	0/0	0/1	0/0	1/4	1/5
		Osseous	0/3	0/2	0/0	0/3	0/8
		Visceral	0/1	0/3	1/1	1/3	2/8
		Total	0/4	0/6	1/1	2/10	3/21
Testosterone propionate, 100 mg 3 × wk i.m.	H. F. Bisel (Cooperative Breast Cancer Group, 1961)	Breast	1,1	0/1	0/0	3/5	4/7
		Osseous	1/2	0/1	1/2	1/5	3/10
		Visceral	0/2	0/0	0/0	2/3	2/5
		Total	2/5	0/2	1/2	6/13	9/22
4-Androstene-$3\alpha,17\beta$-diol, diacetate, 300 mg/wk i.m.		Breast	0/1	1/1	0/1	1/5	2/8
		Osseous	0/0	0/0	1/2	1/4	2/6
		Viscera	0/1	0/1	0/1	0/4	0/7
		Total	0/2	1/2	1/4	2/13	4/21

continued

TABLE I—*continued*

Steroid and dosage	Study	Dominant lesion	Objective regression/Number of patients — Menopausal age: < 1 yr	1–5 yr	5–10 yr	10+ yr	Total
Testosterone propionate, 100 mg 3 × wk i.m.	Olson and Ansfield (1960)	Breast	0/2	0/1	0/1	0/1	0/5
		Osseous	1/3	1/2	0/2	1/3	3/10
		Visceral	0/2	0/2	0/2	1/2	1/8
		Total	1/7	1/5	0/5	2/6	4/23
4-Androstene-3β,17β-diol, diacetate, 300 mg/wk i.m.		Breast	0/0	0/3	0/1	3/4	3/8
		Osseous	0/3	1/3	0/1	1/4	2/11
		Visceral	0/1	0/2	0/1	0/0	0/4
		Total	0/4	1/8	0/3	4/8	5/23
Testosterone propionate, 100 mg 3 × wk i.m.	G. C. Escher (Cooperative Breast Cancer Group, 1961)	Breast	1/1	0/1	0/0	1/2	2/4
		Osseous	0/2	1/2	0/0	0/3	1/7
		Visceral	1/4	0/1	1/2	1/9	3/16
		Total	2/7	1/4	1/2	2/14	6/27
4-Androstene-3,17-dione, 300 mg/wk i.m.		Breast	0/1	0/0	0/0	0/2	0/3
		Osseous	0/1	0/0	0/1	1/3	1/5
		Visceral	0/4	1/1	0/2	0/6	1/13
		Total	0/6	1/1	0/3	1/11	2/21
Testosterone propionate, 100 mg 3 × wk i.m.	Lewison *et al.* (1963)	Breast	0/2	0/0	0/1	1/2	1/5
		Osseous	0/0	0/2	0/0	3/3	3/5
		Visceral	0/3	0/3	0/2	0/2	0/10
		Total	0/5	0/5	0/3	4/7	4/20

Testosterone propionate, 100 mg 3 × wk i.m., with 4,4-dimethyl-17β-hydroxy-5-androsten-3-one, 200 mg 3 × wk i.m.		Breast	0/2	1/1	0/2	2/2	3/7
		Osseous	0/0	0/2	0/1	0/3	0/6
		Visceral	0/2	0/5	0/2	0/3	0/12
		Total	0/4	1/8	0/5	2/8	3/25
Testosterone propionate, 100 mg 3 × wk i.m.	Goldenberg and Hayes (1963)	Breast	0/1	0/1	0/0	0/1	0/3
		Osseous	0/2	0/1	1/1	1/3	2/7
		Visceral	0/0	0/4	1/2	2/4	3/10
		Total	0/3	0/6	2/3	3/8	5/20
Androst-4-en-3-one, 2α,17β-dihydroxy dipropionate 100 mg 3 × wk i.m.		Breast	0/2	1/2	0/2	0/0	1/6
		Osseous	0/1	0/3	0/1	0/3	0/8
		Visceral	0/1	0/2	0/2	0/3	0/8
		Total	0/4	1/7	0/5	0/6	1/22
Androst-4-en-3-one, 16α,17β-dihydroxy dipropionate, 100 mg 3 × wk i.m.		Breast	1/1	0/0	0/1	0/3	1/5
		Osseous	0/2	0/3	0/1	0/3	0/9
		Visceral	0/1	0/3	0/3	0/4	0/11
		Total	1/4	0/6	0/5	0/10	1/25
Testosterone propionate, 100 mg 3 × wk i.m.	Volk *et al.* (1960)	Breast	0/1	0/0	1/1	2/6	3/8
		Osseous	0/0	0/2	0/1	1/4	1/7
		Visceral	0/1	0/0	0/2	2/5	2/8
		Total	0/2	0/2	1/4	5/15	6/23
Progesterone, 3 gm/day p.o.		Breast	0/2	0/1	0/1	0/6	0/10
		Osseous	0/2	0/2	0/0	0/5	0/9
		Visceral	0/3	0/0	0/1	0/3	0/7
		Total	0/7	0/3	0/2	0/14	0/26

continued

TABLE I—*continued*

Steroid and dosage	Study	Dominant lesion	Objective regression/Number of patients: Menopausal age < 1 yr	1–5 yr	5–10 yr	10+ yr	Total
Testosterone propionate, 100 mg 3 × wk i.m.	Goldenberg and Hayes (1959)	Breast	0/1	0/1	0/0	2/4	2/6
		Osseous	0/3	0/0	2/2	2/6	4/11
		Visceral	0/2	0/1	0/1	0/3	0/7
		Total	0/6	0/2	2/3	4/13	6/24
9α-Bromo-11-ketoprogesterone, 300 mg/day p.o.		Breast	0/1	0/1	1/1	2/3	3/6
		Osseous	0/3	0/1	1/3	1/5	2/12
		Visceral	0/2	0/1	0/1	0/3	0/7
		Total	0/6	0/3	2/5	3/11	5/25
Testosterone propionate, 100 mg 3 × wk i.m.	I. Lewin (Cooperative Breast Cancer Group, 1961)	Breast	0/1	0/2	0/0	1/1	1/4
		Osseous	0/3	0/1	0/2	1/5	1/11
		Visceral	0/1	0/2	0/2	0/4	0/9
		Total	0/5	0/5	0/4	2/10	2/24
19-Nor-17α-ethynyltestosterone (norethisterone), 40 mg/day p.o.		Breast	0/2	1/2	0/0	0/1	1/5
		Osseous	0/3	0/0	2/2	1/5	3/10
		Visceral	0/0	1/3	0/3	0/5	1/11
		Total	0/5	2/5	2/5	1/11	5/26
Testosterone propionate, 100 mg 3 × wk i.m.	W. H. Baker and R. M. Kelley (Cooperative Breast Cancer Group, 1961)	Breast	0/1	0/1	1/1	2/7	3/10
		Osseous	0/1	0/1	0/2	1/3	1/7
		Visceral	0/2	0/1	0/1	0/2	0/6
		Total	0/4	0/3	1/4	3/12	4/23

17α-Ethynyl-17-hydroxy-$\Delta^{5(10)}$-estren-3-one (norethynodrel), 40 mg/day p.o.		Breast	0/0	0/1	0/0	2/6	2/7
		Osseous	0/1	0/2	0/0	0/3	0/6
		Visceral	0/1	0/1	0/1	2/4	2/7
		Total	0/2	0/4	0/1	4/13	4/20
Testosterone propionate, 100 mg 3 × wk i.m.	S. G. Taylor, III (Cooperative Breast Cancer Group, 1961)	Breast	0/1	0/2	0/0	2/7	2/10
		Osseous	0/1	0/3	1/1	1/4	2/9
		Visceral	0/0	0/0	0/0	2/4	2/4
		Total	0/2	0/5	1/1	5/15	6/23
Estradiol-17β,3-benzoate, 5 mg 3 × wk i.m.		Breast	0/1	0/2	0/0	1/6	1/9
		Osseous	0/1	0/1	0/1	0/3	0/6
		Visceral	0/2	0/2	0/2	0/4	0/10
		Total	0/4	0/5	0/3	1/13	1/25
Testosterone propionate, 100 mg 3 × wk i.m.	Carter (Cooperative Breast Cancer Group, 1961)	Breast	0/0	2/3	2/2	0/2	4/7
		Osseous	0/2	0/2	0/2	1/3	1/9
		Visceral	0/2	0/3	1/1	1/7	2/13
		Total	0/4	2/8	3/5	2/12	7/29
16α-Estradiol, dipropionate, 5 mg 3 × wk i.m.		Breast	0/0	1/1	0/0	0/5	1/6
		Osseous	0/1	0/4	0/0	0/3	0/8
		Visceral	0/2	0/4	0/1	0/6	0/13
		Total	0/3	1/9	0/1	0/14	1/27
Testosterone propionate, 100 mg 3 × wk i.m.	Dao (Cooperative Breast Cancer Group, 1961)	Breast	0/0	0/1	0/0	2/4	2/5
		Osseous	0/0	0/0	0/3	0/5	0/8
		Visceral	0/1	1/2	0/0	2/9	3/12
		Total	0/1	1/3	0/3	4/18	5/25

continued

TABLE I—*continued*

Steroid and dosage	Study	Dominant lesion	Objective regression/Number of patients: Menopausal age < 1 yr	1–5 yr	5–10 yr	10+ yr	Total
16α-Methylestriol-16β,17β,3-methyl ether, 100 mg/day p.o.		Breast	0/1	0/0	0/1	0/4	0/6
		Osseous	0/0	0/0	0/3	0/5	0/8
		Visceral	0/1	0/1	0/0	4/9	4/11
		Total	0/2	0/1	0/4	4/18	4/25
Testosterone propionate, 100 mg 3 × wk i.m.	Ravdin (Cooperative Breast Cancer Group, 1961)	Breast	0/1	0/1	0/0	1/2	1/4
		Osseous	0/2	0/2	0/3	0/3	0/10
		Visceral	0/2	0/2	2/3	1/4	3/11
		Total	0/5	0/5	2/6	2/9	4/25
Estrololactone, 300 mg/wk i.m.		Breast	0/0	0/0	0/0	0/0	0/0
		Osseous	0/2	0/2	0/3	1/3	1/10
		Visceral	0/1	0/3	0/4	0/3	0/11
		Total	0/3	0/5	0/7	1/6	1/21
Δ^1-Testololactone, 100 mg 3 × wk i.m.	Segaloff *et al.* (1960)	Breast	0/0	1/2	0/0	2/3	3/5
		Osseous	1/2	0/0	1/1	1/5	3/8
		Visceral	0/1	0/2	0/1	1/6	1/10
		Total	1/3	1/4	1/2	4/14	7/23
Testosterone propionate, 100 mg 3 × wk i.m.	Segaloff *et al.* (1962c)	Breast	0/1	0/1	1/2	0/1	1/5
		Osseous	0/0	0/1	0/0	1/4	1/5
		Visceral	1/1	1/2	0/2	0/2	2/7
		Total	1/2	1/4	1/4	1/7	4/17

Testololactone, 100 mg 3 × wk i.m.		Breast	0/1	0/1	0/1	0/2	0/5
		Osseous	0/1	0/3	0/1	0/3	0/8
		Visceral	0/3	0/1	0/0	0/4	0/8
		Total	0/5	0/5	0/2	0/9	0/21
Δ^1-Testololactone, 150 mg/day p.o.		Breast	0/1	0/0	0/0	0/2	0/3
		Osseous	0/0	0/4	0/0	1/4	1/8
		Visceral	2/4	0/2	1/1	1/3	4/10
		Total	2/5	0/6	1/1	2/9	5/21
Testosterone propionate, 100 mg 3 × wk i.m.	Ansfield and Olson (Cooperative Breast Cancer Group, 1961)	Breast	0/0	1/2	0/0	1/3	2/5
		Osseous	0/2	0/3	0/2	0/3	0/10
		Visceral	1/1	1/2	0/1	1/6	3/10
		Total	1/3	2/7	0/3	2/12	5/25
Δ^1-Testololactone, 100 mg 3 × wk i.m.		Breast	0/1	0/2	0/0	0/4	0/7
		Osseous	0/0	0/5	0/0	0/3	0/8
		Visceral	1/2	0/0	0/1	0/4	1/7
		Total	1/3	0/7	0/1	0/11	1/22
Testosterone propionate, 100 mg 3 × wk i.m.	European Breast Cancer Group (1963)	Breast	0/2	1/4	0/4	3/8	4/18
		Osseous	0/3	0/5	0/0	1/5	1/13
		Visceral	0/1	1/5	1/2	1/10	3/18
		Total	0/6	2/14	1/6	5/23	8/49
Δ^1-Testololactone, 100 mg 3 × wk i.m.		Breast	0/1	0/2	0/1	4/13	4/17
		Osseous	0/6	1/4	1/2	1/8	3/20
		Visceral	2/3	0/5	0/5	0/9	2/22
		Total	2/10	1/11	1/8	5/30	9/59

continued

TABLE I—*continued*

Steroid and dosage	Study	Dominant lesion	Objective regression/Number of patients				
			Menopausal age				
			< 1 yr	1–5 yr	5–10 yr	10+ yr	Total
Testosterone propionate, 100 mg 3 × wk i.m.	Segaloff *et al.* (1962a)	Breast	0/0	0/1	0/0	0/0	0/1
		Osseous	0/0	0/1	0/1	0/2	0/4
		Visceral	1/3	0/0	0/1	1/2	2/6
		Total	1/3	0/2	0/2	1/4	2/11
2α-Methyl-5α-dihydrotestololactone, 100 mg 3 × wk i.m.		Breast	0/0	0/1	0/0	0/0	0/1
		Osseous	0/0	0/2	0/3	0/1	0/6
		Visceral	0/4	0/3	1/3	0/4	1/14
		Total	0/4	0/6	1/6	0/5	1/21

TABLE II

STUDIES PRIOR TO AVAILABILITY OF BREAST GROUP PROTOCOL

PART 1

Steroid and dosage	Study	Objective regression/ Number of patients
Methylandrostenediol (17α-methyl-Δ^5-androstene-3β,17β-diol), 100 mg 3 × wk i.m. (2 pts–100 mg/day i.m.)	Segaloff *et al.* (1952a)	2/24
Progesterone, 100 mg 3 × wk i.m.	Segaloff *et al.* (1952a)	2/20
Androstenediol (Δ^5-Androstene-3β,17β-diol), 100 mg 3 × wk i.m.	Segaloff *et al.* (1952b)	0/21
Methyltestosterone (17α-methyl-Δ^4-androsten-3-one, 17β-ol), Oral: 200–300–1000 mg/day p.o.; Buccal: 50–100 mg/day	Segaloff *et al.* (1953)	Oral 3/10 Buccal 5/17 Total 8/27
Conjugated estrogens (equine), 5 mg 3 × day p.o.—28 pts; 10 mg 3 × day p.o.—4 pts; 7.5 mg 3 × day p.o.—1 pt	Segaloff *et al.* (1954b)	11/33 (regressions in soft tissue lesions only)
Dihydrotestosterone (androstanolone) (17β-Hydroxyandrostan-3-one): 100 mg 6 × wk (aqueous suspension); 100 mg 3 × wk (oil suspension) i.m.	Segaloff *et al.* (1955c)	4/15 4/19 Total 8/34
Androstanedione (androstane-3,17-dione), 100 mg 3 × wk i.m.	Segaloff *et al.* (1255b)	0/20
Vinyltestosterone (17α-vinyl-Δ^4-androsten-3-one-17β-ol), 100 mg 3 × wk i.m.	Segaloff *et al.* (1955a)	2/21
Dehydroepiandrosterone (Δ^5-androstene-3β-ol, 17-one), 100 mg 3 × wk i.m.	Segaloff *et al.* (1957b)	0/19
Etiocholanolone (etiocholan-17β-ol-3-one), 100 mg 3 × wk i.m.	Segaloff *et al.* (1957a)	0/18

continued

TABLE II—*continued*

PART 1—*continued*

Steroid and dosage	Study	Objective regression/ Number of patients
Fluoxymesterone (9α-fluoro-17α-methyl-Δ^4-androsten-3-one, 11β,17β-diol), 20 mg/day p.o.	Segaloff *et al.* (1958)	5/23
Epitestosterone propionate (17α-hydroxy-Δ^4-androsten-3-one propionate), 100 mg 3 × wk i.m.	Segaloff *et al.* (1959a)	1/18
Cortisone acetate, parenteral: 10 mg/day—1 pt; 25 mg/day—1 pt; 50 mg/day—1 pt; 100 mg/day—8 pts	Segaloff *et al.* (1954a)	0/11
19-Nortestosterone propionate (17β-hydroxy-Δ^4-estren-3-one propionate), 100 mg 3 × wk i.m.	Segaloff *et al.* (1959b)	2/18
Fluoxymesterone (9α-fluoro-17α-methyl-Δ^4-androsten-3-one, 11β,17β-diol), 20 mg/day p.o.	Kennedy (1957)	14/29
17β-Hydroxy-5α-androstan-3-one (stanolone), 100 mg 3 × wk i.m.	Kennedy (1955)	Breast 2/15 Osseous 4/22 Total 6/37
17α-Hydroxyprogesterone caproate, 250–1000 mg/wk i.m.	Crowley and Macdonald (1962)	1/22
Cortisone, 50 mg/day p.o.	Dao *et al.* (1961)	0/20

TABLE II — *continued*

PART 2

Steroid and dosage	Study	Dominant lesion	Objective regression/ Number of patients				
			Menopausal age				
			< 1 yr	1–5 yrs	5–10 yrs	10+ yrs	Total
Hypophysectomy + hydrocortisone, 30 mg/day p.o. + triiodothyronine 50 μg/day p.o.	Gordan *et al.* (1963)	Breast	0/0	0/1	0/0	0/0	0/1
		Osseous	0/0	1/3	0/0	0/2	1/5
		Visceral	0/4	1/8	1/4	0/5	2/21
		Total	0/4	2/12	1/4	0/7	3/27
Hydrocortisone, 30 mg/day p.o. + triiodothyronine, 50 μg/day p.o.	Gordan *et al.* (1963)	Breast	0/0	0/0	0/0	1/2	1/2
		Osseous	1/2	0/2	0/0	1/3	2/7
		Visceral	0/2	1/3	0/1	1/8	2/14
		Total	1/4	1/5	0/1	3/13	5/23
Prednisone, 30 mg/day p.o. + triiodothyronine, 50 μg/day p.o.	Gordan *et al.* (1963)	Breast	0/1	1/1	0/0	1/2	2/4
		Osseous	0/0	0/2	0/0	2/6	2/8
		Visceral	0/4	1/12	2/3	3/7	6/26
		Total	0/5	2/15	2/3	6/15	10/38
Prednisone, 30 mg/day p.o.	Gordan *et al.* (1963)	Breast	0/0	1/2	0/0	0/1	1/3
		Osseous	0/1	0/3	0/1	0/1	0/6
		Visceral	0/1	0/3	0/3	0/5	0/12
		Total	0/2	1/8	0/4	0/7	1/21
2α-Methyldihydrotestosterone propionate, 100 mg 3 × wk i.m.	Gordan *et al.* (1963)	Breast	0/0	0/1	0/0	0/0	0/1
		Osseous	1/1	0/3	0/0	1/2	2/6
		Visceral	0/3	1/4	1/3	1/9	3/19
		Total	1/4	1/8	1/3	2/11	5/26
6α-Methyl-9α-fluoro-17-acetoxy-21-deoxyprednisolone, 50 mg/day p.o.	Talley *et al.* (1961)	Breast	0/0	2/2	0/1	1/1	3/4
		Osseous	0/2	0/3	0/3	0/1	0/9
		Visceral	1/3	1/2	0/0	0/1	2/6
		Total	1/5	3/7	0/4	1/3	5/19
Fluoxymesterone (9α-fluoro-17α-methyl-Δ^4-androsten-3-one, 11β,17β-diol), 20 mg/day p.o.	Dao and Tan (1962)	Breast	0/1	1/4	1/4	0/4	2/13
		Osseous	0/3	0/4	2/2	2/5	4/14
		Visceral	0/3	1/4	1/2	1/10	3/19
		Total	0/7	2/12	4/8	3/19	9/46

Chapter 6

Androgens

RALPH I. DORFMAN

Methods for the bioassay of androgens were discussed in Volume II of this series. The chapter presents the relative potencies of androgens as determined by these assorted mammalian and ovarian methods when the steroids were administered by the various possible routes.

Relative potencies, in general, vary with the species, the end point, the route of administration, and the multitude of details attending a biological experiment. For this reason the primary presentation deals with relative potencies as determined by carefully defined test procedures.

The first six tables[1] deal with chick comb bioassay procedures. Table I reviews various published papers using the extremely sensitive White Leghorn chick's comb. The test compounds were administered by inunction in ethanol, and the response was judged by the change in weight of the organ. Ofner *et al.* (1962a,b) and Dorfman and Dorfman (1962a) studied eight compounds by inunction of the test compound in ethanol to the comb. The details of the methods, particularly the volume of vehicle, were different. The results indicate that Ofner *et al.* (1962a,b) reported lower relative potency values on five occasions compared to the Dorfman and Dorfman (1962a) values on three. The mean value for one group for all eight comparisons was 18 compared to 124 for the other. It is not unlikely that the differences reported by the two groups may reflect more the results of high error swings than differences in determined relative potency due to the differences in the methods.

Table II deals with the androgenicity of various steroids when inuncted to the chick's comb in corn oil. Except for the substitution of corn oil for ethanol, the method is that used to determine the values reported by Dorfman and Dorfman (1962a) in Table I.

The data listed in Table III were obtained using still another chick comb inunction method, the one published in some detail by Segaloff and Gabbard (1962, 1963) and by Segaloff (1963). This method employs a vehicle consisting of a 1% solution of mineral oil in ether. The comparative androgenicity of various 5β, Δ^{14}, and 7α-methyl substituted steroids are presented.

The Δ^{14} steroids were significantly more active than the corresponding saturated derivatives. 17β-Hydroxy-5α-androstan-3-one had a

[1] For all tables, see pages 245-292.

potency 2.2 times that of testosterone, whereas the Δ^{14} dehydro derivative had a potency of 18.1 times testosterone. In the 5β series, the saturated compound had a relative potency to testosterone of 0.07, which was increased to 0.3 in the steroid possessing the Δ^{14} double bond.

Table III further indicates that removal of carbon 19 results in an increased potency for the following parent steroids: testosterone (increase 100%), 7α-methyltestosterone (50%), testosterone acetate (50%), 7α-dimethyltestosterone (900%), androst-4-ene-3,17-dione (50%), 7α-methylandrost-4-ene-3,17-dione (900%), 17-methyltestosterone (40%), and 7α,17α-dimethyltestosterone (900%).

The addition of the 7α-methyl group to the various molecules produced both increases and decreases in androgenicity. A few examples may be cited as follows, indicating the parent compound and the change in relative potency, in percentage, by addition of the 7α-methyl group: methyltestosterone, +100; androst-4-ene-3,17-dione, −88; 17-methyl-19-nortestosterone, +1300, and testosterone, 0.

The relative potencies of a limited number of steroids have been determined using the subcutaneous injection route and the chick's comb as the end point. Table IV deals with the activity determination when the compounds were injected in an oil vehicle, and Table V summarizes the compounds studied by the chick's comb test using an aqueous Tween suspension as the injection vehicle. Table VI deals with the relative potency of the steroids when administered admixed with the chick food.

In the past, the capon's comb has been used as an important indicator for androgenic activity, and various injection and inunction methods have been employed. Selye (1943) has summarized this older literature; Tables VII–X deal with this material. Tables VII and VIII deal with injection methods, and Tables IX and X indicate relative potencies of various compounds when applied directly to the capon's comb.

The method of Eisenberg and Gordan (1950) has been used to evaluate the androgenicity of one compound by gavage (Table XI) and of a rather long list of compounds by subcutaneous injection (Table XII). In either case, the androgenicity was assessed on the basis of the seminal vesicle response in the castrated rat when the test material was administered over a 21-day period.

An adrenalectomized-castrated rat test has been employed to determine whether the tricyclic compound 2-acetyl-7-oxo-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydrophenanthrene possesses androgenic activity in the absence of all steroid hormone-forming tissues. Since both the seminal vesicles and prostate were stimulated by this compound in this

test, it was concluded that this synthetic compound is, in fact, an androgen (Dorfman, 1960b).

A preputial gland assay in the hypophysectomized rat has been used to establish relative potencies of three C_{18} steroids with single oxygen functions. Relatively highly active androgens are possible in steroids which lack the oxygen function (Sydnor, 1958) (Table XIII). In these mono oxygenated compounds, a 17β-hydroxy function appears to be far more important to facilitate high activity than a 17-ketone group. Thus, 3β-fluoro-5α-androstan-17-one was less than one-sixth as active as 3β-fluoro-5α-androstan-17β-ol.

Mathieson and Hays (1945) suggested a method for the assay of androgens using a single injection into a castrated rat and using the weight response of the seminal vesicle as a means of judging relative potency. A single example is presented in Table XIV. A bone maturation test in mice is indicated in Table XV. In this test both dehydroepiandrosterone sulfate and the 11β-hydroxyandrost-4-ene-3,17-dione showed particularly high relative potencies (Howard, 1962).

The intact rat has been employed as a test animal for the detection of androgens, and some typical data are presented in Table XVI based on the assays of Butenandt and Hanisch (1935) and Tscherning (1936). These data have been previously summarized by Selye (1943). Tables XVII and XVIII list references to various androgens without attempting to judge relative potencies where the rat seminal vesicle and prostate tests were employed, respectively.

As part of the bioassay procedure using the castrated male in parabiosis with the intact female rat, the test compound is studied for androgenic activity using the prostate and seminal vesicle end points. Typical data are represented in Table XIX (prostate) and XX (seminal vesicles) for tests where the compounds were administered by subcutaneous injection.

Within the past few years, many investigators have employed the method of Hershberger *et al.* (1953) to evaluate the relative potencies of test compounds using both the seminal vesicle and prostate responses when the steroids were administered either by gavage or by subcutaneous injection. The results of these assays are summarized in Tables XXI through XXIV. The first two tables in this series deal with subcutaneously injected steroids and Tables XXIII and XXIV deal with a similar list of steroids administered by gavage.

Table XXV is a collection of androgen bioassay reports in which the test was not specified. Table XXVI is a summary of relative potencies in which the Hershberger *et al.* (1953) test was employed, but some uncertainties exist as to whether the seminal vesicle or prostate end

points were used to evaluate the relative potencies, or perhaps both end points were used.

In the course of various studies on the relationship between structure and function, a group of steroids frequently related to biologically active substances have been synthesized which, even at relatively high doses, are inactive. Table XXVII summarizes various steroids which have been judged to be inactive in a chick comb assay with absolute ethanol inunction. Parallel studies are reported for inactive compounds in castrated rat assays in Table XXVIII.

Table XXIX presents those steroids which have been assayed by two or more inunction methods. The comparison between the Ofner *et al.* (1962a,b) and Dorfman and Dorfman (1962a) studies has been discussed in an earlier section of this chapter. The data of Segaloff (1963) and those of Segaloff and Gabbard (1962, 1963) are here added. Androst-4-ene-3,17-dione and 17β-hydroxy-5α-androstan-3-one were assayed by all methods, and in both cases relative potency agreements were found with methods A and C; method B yielded what appears to be a significantly lower value in one case, and a higher value in the other. 5α-Androstane-3,17-dione was some four times more active by method C as when method A was employed (Table XXIX).

Four compounds were studied by ethanol inunction by Ofner *et al.* (1962a,b) which were also studied by Dorfman and Dorfman (1962a) by subcutaneous injection with the test material contained in a Tween suspension. The results are included in Table XXX for easy comparison, they indicate a reasonable correspondence in relative potency for the three steroids, 19-nortestosterone, dehydroepiandrosterone, and androst-4-ene-3β,17β-diol, but in the case of androst-4-ene-3,17-dione more than a tenfold difference was found. A similar lack of correspondence was found for the relative potency of androst-4-ene-3,17-dione as determined by Dorfman and Dorfman (1962a) by ethanol inunction as compared to the Tween suspension value obtained by the subcutaneous route.

Some fourteen steroids were studied by both corn oil inunction and ethanol inunction methods (Ofner *et al.*, 1962a,b) (Table XXXI). The mean relative potencies by the two methods were not seriously different, 0.9 and 1.1, respectively. However, this analysis tends to cover up some rather wide discrepancies: for example, adrenosterone was fifteen times more effective by the ethanol inunction technique; 5α-androstane-3,17-dione was ten times more comb-growth stimulating by the ethanol method; and 11β-hydroxyandrost-4-ene-3,17-dione was six times more effective by the corn oil method.

For five androgens, the corn oil inunction (Dorfman and Dorfman, 1962a) relative potency, and the ethanol inunction relative potency (Dorfman and Dorfman, 1962a) were determined and again, mean overall relative potencies of 1.2 and 1.4, were found for the two methods. Again, 5α-androstane-3,17-dione was much less active by the corn oil inunction method; in this instance the difference was sixfold (Table XXXI).

Table XXXII lists the data which compare the data obtained by the corn oil chick comb inunction method with that of the Tween suspension subcutaneous injection method. Dehydroepiandrosterone gave essentially the same relative potency, whereas 19-nortestosterone was some twice as potent by inunction. The striking difference was found for androst-4-ene-3,17-dione, which was about ten times more potent by the corn oil inunction methods.

Table XXXIII deals with the relative potencies of various androgens as determined by ethanol inunction and corn oil injection. One striking difference was observed, and that was for the steroid androst-4-ene-3,17-dione, which was one-sixth as active by injection compared to the Dorfman and Dorfman (1962a) ethanol inunction data and less than one-tenth as potent (relative potency of testosterone) as by the Ofner *et al.* (1962a,b) method.

The relative potency of androst-4-ene-3,17-dione was seven to eight times greater by corn oil inunction than when injected in the same vehicle (Table XXXIV). The reduced steroid, however, 17β-hydroxy-5α-androstan-3-one, was 3.7 times as active as testosterone by injection in corn oil and almost as potent compared to testosterone when inuncted.

The comparative relative potencies of various androgens inuncted in corn oil to the chick comb and incorporated in the food are illustrated in Table XXXV. Striking decreases in relative potencies in the food were found for three 3β-hydroxy steroids: dehydroepiandrosterone, decrease from 0.55 to 0.07; 17α-methylandrost-5-ene-3β,17β-diol, from 0.65 to 0.05; and epiandrosterone, from 0.23 to 0.06. 17β-Hydroxy-5α-androstan-3-one had a relative potency of 1.94 by inunction, but only 0.14 in the food, whereas the closely related 5α-androstane-3,17-dione had essentially similar relative potencies by the two routes of 0.18 by inunction and 0.24 in the food. Adrenosterone was far more potent by the oral route, with a relative potency of 0.32 compared to 0.02 by inunction in corn oil.

The data of Table XXXVI are quite similar to those previously discussed from Table XXXV. Again, the 3β-hydroxy steroids were far less active by the oral route than by the ethanol inunction method. This intense decrease in relative potency was shown by dehydroepi-

androsterone from 0.59 and 0.66 for the ethanol methods to 0.07 for the oral route. For 17α-methylandrost-5-ene-3β,17β-diol the change was from 0.87 to 0.05 and for epiandrosterone from 0.67 to 0.06. Both 17β-hydroxy-5α-androstan-3-one and 5α-androstane-3,17-dione were far less active, compared to testosterone, by the oral route than by the ethanol inunction method of testing.

The comparative relative comb growth activity of androgens in corn oil or incorporated in the food is presented in Table XXXVII. The two Δ^4-diols displayed relative potencies close to unity under all circumstances. Androst-4-ene-3,17-dione was one-third as active by corn oil injection as when admixed with the food, while 17β-hydroxy-5α-androstan-3-one was almost 15 times more potent by the injection route as compared to testosterone.

The synthesis of a wide variety of *D*-homo steroids corresponding to highly active androgens has permitted the study of their relative potency by a capon's comb method (Heusser *et al.*, 1954) (Table XXXVIII). No simple rule of change in biological activity could be observed; rather instances of no change, increase in activity, and decrease in relative potency were found. The *D*-homo analog of the inactive 17β-methylepitestosterone was also inactive. The potency of both testosterone and epiandrosterone were decreased by one-half and the potencies of androsterone, 17β-hydroxy-5α-androstan-3-one and 5α-androstane-3,17-dione were unchanged. The androgenicity of 17α-methyltestosterone and 5α-androstane-3β,17α-diol were doubled, that of 5α-androstane-3β,17β-diol tripled, and epiandrosterone increased some fivefold.

The relative potencies of various androgens by different injection and inunction tests in the capon are illustrated in Table XXXIX. The relative potency by injection of 17β-hydroxy-5α-androstan-3-one was 0.6 and 1.0. Equally good agreement for the injection studies was found for 5α-androstane-3α,17β-diol at 0.6 and 0.6, androst-4-ene-3,17-dione at 0.13 and 0.14, 5α-androstane-3,17-dione at 0.13 and 0.09, androst-5-ene-3β,17β-diol at 0.03 and 0.02, epiandrosterone at 0.02 and 0.02, and androsterone at 0.15 and 0.16. The inunction values tended to be higher. Epiandrosterone was five times more active on a relative basis to testosterone. In the case of androst-4-ene-3,17-dione, the relatively increased potency compared to testosterone by inunction was seven times for one set of data and almost twenty times by the other method (Table XXXIX).

The comparative relative potencies of subcutaneously injected androgens in a castrated rat assay using the seminal vesicle weight end point are presented in Table XL, and good agreement was found for

the relative potencies of the three compounds as determined by the two different assay procedures.

Table XLI lists the relative potency of a few androgens studied by various investigators in a castrated rat assay using prostate weight as the end point.

Table XLII illustrates the striking difference found for esters of diverse acid groups. The most active ester was the dichloroacetate of testosterone, which was 1030% and 769% more active than testosterone on the ventral prostate and seminal vesicle end points, respectively. Testosterone acetate was essentially as active as the free compound, while the propionate and fluorochloro esters were significantly more active on all parameters. The methyl ether was distinctly less active than free testosterone with all three end points.

The relative androgenic potencies of 2-substituted derivatives of 5α-androst-2-en-17β-ol are presented in Table XLIII. The compounds are listed on the basis of the relative androgenic activity (mean of seminal vesicle and prostate weight responses) The order of activity for the C-2 substituents listed in decreasing order of activity was. $CH_3 > CH_2 > CH_2OH > CHO > COOH$.

The effect of introducing various double bonds into ring A of the 5α-androstan-17β-ol molecule is illustrated in Tables XLIV and XLV. Steroids having a formyl grouping at position 2 and a single double bond (Δ^2) were generally more active than the saturated compounds or derivatives with an additional unsaturated linkage. 2-Methyl-Δ^2 steroid was more active than a 2-methylene compound which possesses an exocyclic double bond. Formation of the 17β-acetate decreased the activity in this case, probably due to the difficulties encountered in clearing the ester to form the free steroid as is likely necessary for expression of biological activity.

Table XLVI lists five steroidal ring A olefins. These compounds possess only the 17β-alcoholic function, either free or in the form of the acetate, and double bonds in ring A of the nucleus as indicated. The most active compounds were 5α-androst-2-en-17β-ol and 5α-androst-1-en-17β-ol, both 60% as active as testosterone. The order of activity of the Δ^3 and $\Delta^{1,3}$ derivatives of the 5α-androstan-17β-ol parent compound was found to be 40% as androgenic as testosterone.

The influence of various C-10 substituents is given in Table XLVII. The removal of the angular methyl group of testosterone results in the formation of 19-nortestosterone with a marked decrease in androgenic potency. Substitution of the 10-methyl by a 10-ethyl grouping decreased the activity about twentyfold. The decrease in androgenic activity was not as significant in the presence of a 10-vinyl group derivative of 19-

nortestosterone, which was 82% as androgenic as the standard, testosterone.

Table XLVIII deals with the effect of different groups introduced into 17α-methyl-5α-androstan-17β-ol; the assays were performed by gavage. The Δ^2 compound, 17α-methyl-5α-androst-2-en-17β-ol, and the 2-nitrolo derivative had a high androgenic activity of 170% and 130% of methyltestosterone. 2-Methylene-17α-methyl-5α-androst-2-en-17β-ol was about as active as the standard, and the remaining three steroids were significantly less active.

Activity of the 3-Alcohols

Among the naturally occurring steroid hormones and metabolites, the androgens are unique in that 3-hydroxy-5α-androstanes exhibit high hormonal activity, the 3α-hydroxy (axial) compounds being highly active, while the 3β-hydroxy (equatorial) compounds are much weaker androgens. It is known from both chemical and microbiological work that the 3α-alcohols are oxidized more readily to the corresponding 3-ketone than are the 3β-alcohols in accordance with the thermodynamic prediction. It is further known that enzymic oxidation of 3-hydroxy-5α-androstanes occurs in mammalian tissue, in particular in the liver. This suggests the possibility that, *in vivo*, these compounds are oxidized in various degrees to the even more potent androgens, the 3-keto-5α-androstanes, and that, in fact, the 3-alcohols may be androgenically inactive per se.

To test this hypothesis, Ringold *et al.* (1961) synthesized 3α-deutero-17α-methyl-5α-androstane-3β,17β-diol (I) by the lithium aluminum deuteride reduction of 17α-methyl-5α-androstan-17β-ol-3-one (III) and assayed this deuterated steroid in the castrated rat in parallel with the corresponding 3α-hydrogen-17α-methyl-5α-androstane-3β,17β-diol (II). Should the activity of these compounds depend on oxidation to the 3-ketone with loss of the 3α-hydrogen or 3α-deuterium as the rate-determining step, then, in accord with the primary deuterium isotope effect, the 3α-deutero compound (I) should be oxidized more slowly and consequently would be expected to exhibit lower androgenic activity than the 3α-hydrogen compound (II).

Male albino rats were castrated at 22–24 days of age, and beginning on the day of surgery, the compounds were injected subcutaneously once daily for seven consecutive days. The vehicle was an aqueous suspending medium consisting of sodium chloride (0.9%), polysorbate 80 (0.4%), carboxymethylcellulose (0.5%), and benzyl alcohol (0.9%), and the daily dose was contained in 0.5 ml. Twenty-four hours after the last injection, the rats were sacrificed with ether and the ventral

prostate, seminal vesicles, and levator ani were removed and weighed wet after blotting.

Compound II was 3.36 [+1.64; −1.10 ($P=0.95$)] times more active than the deutero compound (I), on the basis of the ventral prostate, and 4.65 [+1.67; −1.25 ($P=0.95$)] times more active on the basis of response of the seminal vesicles, demonstrating that in the rat oxidation to the 3-ketone is an important, if not the sole, factor contributing to androgenicity of 3β-hydroxyandrostanes.

In the chick comb assay, by local inunction, no significant difference could be established between the activities of I, II, and III. When the potency of compound II was assigned a value of 1, the relative potency of compound I was 1.03 [+0.51; −0.33 ($P=0.95$)] and that of compound III was 1.04 [+0.46; −0.32 ($P=0.95$)]. This indicates that in this species either the 3β-hydroxyandrostanes are active per se or more likely that oxidation to the 3-ketone in the comb is rapid and essentially complete.

It was of interest that we could not establish any difference in androgenic activity between testosterone and 17α-deuterotestosterone in the castrated rat by either the oral or subcutaneous route, nor could Bollinger and Wendler (1959) increase the anti-inflammatory activity of hydrocortisone by conversion to the 11α-deutero analog.

Substitution of Oxygen for Carbon at Postion 2

Pappo and Jung (1962) argued that "the substitution of a hetero atom for a 2-methylene group in steroid hormones should lead to products useful in the elucidation of the mechanism of biological action of these compounds." They further pointed out that such replacement should not change the shape of the molecule and should not alter the 4,5 double bond. However, the replacement of the 2-methylene group by oxygen changes the 3-ketone to a lactone group Pappo and Jung (1962) did, in fact, synthesize the 2-oxa-17α-methyltestosterone, which proved to be about one-fifth as androgenic as 17α-methyltestosterone in the castrated rat seminal vesicle assay by injection

Role of 19-Nor Steroids (Segaloff, 1963)

Segaloff used a 1% mineral oil solution in ether as a vehicle for various androgens for inunction to the chick's comb. Graphic analysis of his data bears out his contention that in each of eight examples removal of the 19-methyl group did, in fact, increase the relative potency of the steroid by factors of 50–900%. Among these examples are testosterone, androst-4-ene-3,17-dione, and methyltestosterone, and on the basis of these facts, Segaloff (1963) advanced the thesis that "the 19-nor andro-

gens may well be the active androgens at the end organ, that they in turn give rise to the estrogens, and that testosterone merely serves as a precursor of the active 19-nor steroids."

Influence of the 7α-Methyl Group

Campbell *et al.* (1963) have shown that the introduction of the 7α-methyl group into the 17α-methyl-19-nortestosterone molecule increases the androgenicity orally in the rat. A threefold increased oral androgenicity was reported upon the introduction of the same group into methyltestosterone.

The data of Segaloff (1963) on the influence of the 7α-methyl group have been calculated and appear to indicate a rather wide variation in change in biological activity depending upon the compound, the end point, and the route of administration. The data of Segaloff (1963) are in excellent agreement with those of Campbell *et al.* (1963), as Table XLIX shows. In only one single case of the introduction of the 7α-methyl group, that is into the 17α-methyl-19-nortestosterone molecule was this followed by a large and consistent increase in biological activity. By the chick comb assay procedure a fourteenfold increase was observed; in the castrate rat by gavage, the prostate end point registered an increase of one-hundredfold and the seminal vesicles an eighteenfold increment. In the castrate rat by injection, the seminal vesicles indicated again an enormous increase of one-hundredfold and the ventral prostate a change of sixtyfold.

In four of the five tests, 7α-methylandrost-4-ene-3,17-dione was less active than the parent compound.

Δ^{14}-Group (Segaloff and Gabbard, 1963)

This unsaturated group in the testosterone molecule caused a fourfold increase in testosterone, about a sevenfold increase in 17β-hydroxy-5α-androstan-3-one, and a fifteenfold increase in the corresponding 5β-reduced compound. However, there was a decreased androgenicity on the basis of the castrated mouse seminal vesicle test and the castrated rat prostate test when the Δ^{14} group was inserted into the testosterone or 17β-hydroxy-5α-androstan-3-one molecules.

TABLE I

RELATIVE POTENCY OF ANDROGENS BY A CHICK COMB INUNCTION TEST (ETHANOL)

Steroid	Relative potency (testosterone = 1) (95% confidence limits)	References
Androst-3,5-dien-17β-ol	0.1	Dorfman *et al.* (1962a)
4-Methylestra-1,3,5(10)-trien-17β-ol	0.03	Dorfman *et al.* (1962a)
3α-Deutero-17α-methyl-5α-androstane-3β,17β-diol	1.03 (+0.51; −0.33)	Dorfman *et al* (1962a)
17α-Methyl-5α-androstan-3-one	1.04 (+0.46; −0.32)	Dorfman *et al.* (1962a)
Androst-4-ene-3,17-dione	1.21 (1 07–1.35)	Dorfman and Dorfman (1962a)
	2 60	Ofner *et al.* (1962a,b)
17α-Methyltestosterone	1.93 (162–238)	Dorfman and Dorfman (1962a)
	2.31	Ofner *et al.* (1962a,b)
17β-Hydroxyandrosta-1,4-dien-3-one	0.12 (0.08–0.19)	Dorfman and Dorfman (1962a)
2α,17α-Dimethyl-17β-hydroxy-5α-androstan-3-one	0.63 (0.51–0.73)	Dorfman and Dorfman (1962a)
2-Methyl-17β-hydroxy-5α-androst-1-en-3-one	1.00 (0.72–1.39)	Dorfman and Dorfman (1962a)
10β-Fluoro-17β-hydroxy-5β-19-norandrostan-3-one	1.29 (1.04–1.63)	Dorfman and Dorfman (1962a)
2-Hydroxymethylene-17α-methyl-5α-androstan-3-one	0.04 (0.03–0.06)	Dorfman and Dorfman (1972a)
17α-Methyl-17β-hydroxyandrosta-1,4-dien-3-one	0.13 (0.10–0.17)	Dorfman and Dorfman (1962a)
2α-Methyl-17β-hydroxyandrost-4-en-3-one	0.38 (0.32–0.45)	Dorfman and Dorfman (1962a)
17β-Hydroxy-5α-androstan-3-one	2.28 (1.51–3.44)	Dorfman and Dorfman (1962a)
	1.07	Ofner *et al.* (1962a,b)
Androsterone	1.00 (0.85–1.17)	Dorfman and Dorfman (1962a)
Androsta-4,9(11)-diene-3,17-dione	0.29 (0.24–0.33)	Dorfman and Dorfman (1962a)
6α-Fluoro-17α-ethynyltestosterone	0.04 (0.03–0.05)	Dorfman and Dorfman (1962a)

continued

TABLE I—*continued*

Steroid	Relative potency (testosterone = 1) (95% confidence limits)	References
17β-Hydroxy-5α-19-norandrost-4-en-3-one	1.18 (0.89–1.57)	Dorfman and Dorfman (1962a)
17α-Ethylandrost-3β,17β-diol	0.10 (0.07–0.15)	Dorfman and Dorfman (1962a)
Dehydroepiandrosterone	0.59 (0.26–0.98)	Dorfman and Dorfman (1962a)
	0.66	Ofner *et al.* (1962a,b)
17α-Ethyl-17β-hydroxy-5α-19-norandrostan-3-one	0.08 (0.05–0.12)	Dorfman and Dorfman (1962a)
5α-Androstane-3,17-dione	1.15 (0.90–1.46)	Dorfman and Dorfman (1962a)
	1.82	Ofner *et al.* (1962a,b)
5α-Androstane-3,11,17-trione	0.47 (0.39–0.55)	Dorfman and Dorfman (1962a)
17α-Methyl-17β-hydroxy-5α-androstano(2,3-*b*)-thiazole	0.01 (0.006–0.014)	Dorfman and Dorfman (1962a)
17α-Ethyl-19-nortestosterone	2.70 (2.00–3.70)	Dorfman and Dorfman (1962a)
17α-Methyl-17β-hydroxy-5α-androstane (2,3-*b*)-2′-methylthiazole	0.20 (0.14–0.29)	Dorfman and Dorfman (1962a)
Androst-5-ene-3β,17β-diol	1.05 (0.74–1.56)	Dorfman and Dorfman (1962a)
	0.36	Ofner *et al.* (1962a,b)
2-Hydroxymethylene-17β-hydroxy-5α-androstan-3-one	0.05 (0.03–0.09)	Dorfman and Dorfman (1962a)
17α-Methyl-17β-hydroxy-5α-19-norandrostan-3-one	0.52 (0.35–0.78)	Dorfman and Dorfman (1962a)
17α-Ethyl-17β-hydroxy-19-norandrostan-3-one	0.34 (0.23–0.51)	Dorfman and Dorfman (1962a)
2,17-Dimethyl-17β-hydroxy-5α-androstan-3-one azine	0.44 (0.27–0.78)	Dorfman and Dorfman (1962a)
2α-Hydroxymethyl-17β-hydroxy-5α-androstan-3-one	0.13 (0.08–0.20)	Dorfman and Dorfman (1962a)
2-Methyl-5α-androst-2-en-17β-ol	0.16 (0.12–0.27)	Dorfman and Dorfman (1962a)
2-Hydroxymethyl-5α-androst-2-en-17β-ol	0.05 (0.02–0.17)	Dorfman and Dorfman (1962a)
2-Hydroxymethylene-17α-methyl-17β-hydroxy-5α-androstan-3-one	0.05 (0.03–0.07)	Dorfman and Dorfman (1962a)
2α-Hydroxymethyl-5α-androstan-17β-ol-diacetate	0.13 (0.08–0.20)	Dorfman and Dorfman (1962a)

TABLE I— *continued*

Steroid	Relative potency (testosterone = 1) (95% confidence limits)	References
11β-Hydroxy-17α-methyltestosterone	< 0.10	Ofner *et al.* (1962a,b)
9α-Fluoro-11β-hydroxy-17α-methyltestosterone	< 0.10	Ofner *et al.* (1962a,b)
Pregna-4,16-diene-3,20-dione	0 08	Ofner *et al.* (1962a,b)
11 Keto-17α-methyltestosterone	0.12	Ofner *et al.* (1962a,b)
11-Ketotestosterone	0.09	Ofner *et al.* (1962a,b)
17α-Vinyltestosterone	0 11	Ofner *et al.* (1962a,b)
Androsta-1,4-diene-3,17-dione	0.42	Ofner *et al.* (1962a,b)
17β-Hydroxy-5α-androst-1-en 3-one	0.48	Ofner *et al.* (1962a,b)
17β-Hydroxyandrosta 1,4-dien-3-one	0.13	Dorfman and Dorfman (1962a)
	0.30	Ofner *et al* (1962a,b)
19-Nortestosterone	0.86	Ofner *et al.* (1962a,b)
Androsterone	2 40	Ofner *et al.* (1962a,b)
Adrenosterone	0.29	Ofner *et al.* (1962a,b)
Testosterone propionate	3.80	Ofner *et al.* (1962a,b)
Epiandrosterone	0.67	Ofner *et al.* (1962a,b)
11β-Hydroxyandrosten 4-ene-3,17 dione	0.08	Ofner *et al.* (1962a,b)
	< 0 05	Dorfman and Dorfman (1962a)
Androst-4-ene 3β,17β-diol	1 17	Dorfman and Dorfman (1962b)
	0.76	Ofner *et al.* (1962a,b)
5α-Androst-1-ene-3,17-dione	1.15	Ofner *et al.* (1962a,b)
17α-Methylandrost-5-ene-3β,17β-diol	0.87	Ofner *et al.* (1962a,b)
9α-Bromo-11β-hydroxyandrost-4-ene-3,17-dione	Active	Lenhard and Bernstein (1955)
9β,11β-Oxidoandrost-4-ene-3,17-dione	Active	Lenhard and Bernstein (1955)
17α-Methylandrost-4-ene-3β,17β-diol	1 0[a]	Bernstein *et al.* (1957)

[a] Methyltestosterone standard.

TABLE II

RELATIVE POTENCY OF ANDROGENS BY A CHICK COMB INUNCTION TEST (OIL)[a]

Steroid	Relative potency (testosterone = 1) (95% confidence limits)
Androst-4-ene-3β,17-diol	1.17 (0.99–1.41)
Androsterone	1.64 (1.43–1.80)
Androst-4-ene-3,17-dione	1.78 (1.56–2.05)
Adrenosterone	0.22 (0.16–0.32)
17β-Hydroxy-5α-androstan-3-one	1.94 (1.50–2.47)
Androsta-1,4-diene-3,17-dione	1.78 (1.41–2.36)
11β-Hydroxyandrost-4-ene-3,17-dione	0.46 (0.32–0.63)
Dehydroepiandrosterone	0.54 (0.38–0.70)
5α-Androstane-3,17-dione	0.18 (0.16–0.21)
19-Nortestosterone	0.72 (0.63–0.82)
Epiandrosterone	0.23 (0.18–0.30)
Pregna-4,16-diene-3,20-dione	0.12 (0.09–0.17)
17α-Methylandrost-5-ene-3β,17β-diol	0.65 (0.53–0.78)
17β-Hydroxyandrosta-4,7-dien-3-one	0.36 (0.29–0.43)
17α-Methyltestosterone	1.45 (1.25–1.67)
17α-Methyl-17β-hydroxy-5α-androst-1-en-3-one	1.58 (1.15–1.86)
5α-Androst-1-ene-3,17-dione	2.06 (1.76–2.40)
9α-Fluoro-11β-hydroxy-17α-methyltestosterone	0.04 (0.02–0.06)
11β-Hydroxy-17α-methyltestosterone	0.04 (0.02–0.06)
11-Keto-17α-methyltestosterone	0.06 (0.04–0.08)

[a] Data of Dorfman and Dorfman (1962a).

TABLE III

RELATIVE POTENCY OF ANDROGENS BY A CHICK COMB INUNCTION TEST (ETHER)

Steroid	Relative potency (testosterone = 1) (95% confidence limits)	References
17β-Hydroxyandrosta-4,14-dien-3-one	4.1 (3.8–4.3)	Segaloff and Gabbard (1962, 1963)
17β-Hydroxy-5α-androstan-3-one	2.2 (1.6–3.1)	Segaloff and Gabbard (1962, 1963)
17β-Hydroxy-5α-androst-14-en-3-one	18.1 (11.1–30.0)	Segaloff and Gabbard (1962, 1963)
17β-Hydroxy-5β-androstan-3-one	0.02 (0.01–0.03)	Segaloff and Gabbard (1962, 1963)
17β-Hydroxy-5β-androst-14-en-3-one	0.3 (0.2–0.67)	Segaloff and Gabbard (1962, 1963)
17β-Hydroxy-5α-androstan-3-one	2.3 (1.5–3.7)	Segaloff and Gabbard (1962, 1963)
17β-Hydroxy-5β-androstan-3-one	0.07 (0 04–0.10)	Segaloff and Gabbard (1962, 1963)
5α-Androstane-3,17-dione	2.1 (1.6–2.7)	Segaloff and Gabbard (1962, 1963)
Androst-4-ene-3,17-dione	1.3 (0.8–2.2)	Segaloff and Gabbard (1962, 1963)
5α-Androstane	Active	Segaloff and Gabbard (1960)
8-Isotestosterone	0.4	Djerassi *et al.* (1956)
19-Nortestosterone	2.0	Segaloff (1963)[a]
7α-Methyltestosterone	1.0	Segaloff (1963)
7α-Methyl-19-nortestosterone	1.5	Segaloff (1963)
Testosterone acetate	0.4	Segaloff (1963)
19-Nortestosterone acetate	0.6	Segaloff (1963)
7α-Methyltestosterone acetate	0.1	Segaloff (1963)
7α-Methyl-19-nortestosterone acetate	1.0	Segaloff (1963)
Androst-4-ene-3,17-dione	0.4	Segaloff (1963)
19-Norandrost-4-ene-3,17-dione	0.6	Segaloff (1963)
7α-Methylandrost-4-ene-3,17-dione	0.05	Segaloff (1963)
7α-Methyl-19-norandrost-4-ene-3,17-dione	1	Segaloff (1963)
17-Methyltestosterone	5	Segaloff (1963)
17-Methyl-19-nortestosterone	7	Segaloff (1963)
7α,17α-Dimethyltestosterone	10	Segaloff (1963)
7α,17α-Dimethyl-19-nortestosterone	100	Segaloff (1963)

[a] Relative potencies cited from Segaloff (1963) were estimated graphically.

TABLE IV

RELATIVE POTENCY OF ANDROGENS BY A CHICK SUBCUTANEOUS INJECTION TEST (CORN OIL)

Steroid	Relative potency (testosterone = 1) (95% confidence limits)	References
Androst-4-ene-3,17-dione	0.21 (0.16–0.27)	Dorfman and Dorfman (1962b)
17β-Hydroxy-5α-androstan-3-one	3.70 (3.18–4.22)	Dorfman and Dorfman (1962b)
Testosterone propionate	3.95 (3.39–4.51)	Dorfman and Dorfman (1962b)
Androst-4-ene-3α,17β-diol	1.01 (0.70–1.38)	Dorfman *et al.* (1962b)
Androst-4-ene-3β,17β-diol	0.99 (0.76–1.26)	Dorfman *et al.* (1962b)

TABLE V

RELATIVE POTENCY OF ANDROGENS BY A CHICK SUBCUTANEOUS INJECTION TEST (TWEEN)[a]

Steroid	Relative potency (testosterone = 1) (95% confidence level)
Androst-4-ene-3,17-dione	0.17 (0.13–0.22)
Androst-5-ene-3β,17β-diol	0.49 (0.32–0.71)
Dehydroepiandrosterone	0.65 (0.51–0.83)
19-Nortestosterone	0.35 (0.30–0.40)
Androst-4-ene-3β,17β-diol	0.49

[a] Data of Dorfman and Dorfman (1962b).

TABLE VI

RELATIVE POTENCY OF ANDROGENS BY A CHICK COMB TEST (ANDROGENS IN FOOD)

Steroid	Relative potency (testosterone = 1)	References
Androst-4-ene-3,17-dione	0.71	Dorfman and Dorfman (1963)
17α-Methyltestosterone	0.63	
19-Nortestosterone	0.76	
Adrenosterone	0.32	
Dehydroepiandrosterone	0.07	
17α-Methylandrost-5-ene-3β,17β-diol	0.05	
17β-Hydroxy-5α-androstan-3-one	0 14	
Epiandrosterone	0.06	
5α-Androstane-3,17-dione	0.24	
11β-Hydroxyandrost-4-ene-3,17-dione	0 43	
11α-Hydroxyandrost-4-ene-3,17-dione	0 57	
Androst-4-ene-3β,17β-diol	1.24	Dorfman *et al.* (1962b)
Androst-4-ene-3α,17β-diol	0.97	

TABLE VII

RELATIVE POTENCY OF ANDROGENS BY A CAPON'S COMB INJECTION TEST[a]

Steroid	Relative potency (testosterone = 1)
17β-Hydroxy-5α-androstan-3-one	1.0
17α-Methyl-17β-hydroxy-5α-androstan-3-one	1.0
D-Homo-17β-hydroxy-5α-androstan-3-one	0 5
5α-Androstane-3α,17β-diol	0.6
Methyltestosterone	0.6
Testosterone methyl carbonate	0.5
Testosterone phenyl carbonate	0.5
17α-Methyl-5α-androstane-3α,17β-diol	0.5
Androst-5-ene-3α,17β-diol	0.5
17α-Ethyl-5α-androstane-3α,17β-diol	0.3
D-Homo-3β-hydroxy-5α-androstan-17-one	0.17
Androsterone	0.15

continued

TABLE VII—*continued*

Steroid	Relative potency (testosterone = 1)
17β-Hydroxyandrost-5-en-17-one	0.15
Androst-4-ene-3,17-dione	0.13
5α-Androstane-3,17-dione	0.13
17β-Hydroxyandrosta-4,6-dien-3-one	0.06
Dehydroepiandrosterone	0.06
Androst-4-ene-6,17-dione	0.06
3β-Hydroxyandrost-4-en-17-one	0.06
Androst-3,5-dien-17-one	0.04
Epitestosterone	0.04
Androst-5-ene-3β,17β-diol	0.03
5α-Androstane-3β,17β-diol	0.03
17α-Methylandrost-5-ene-3β,17β-diol	0.03
17α-Ethylandrost-5-ene-3β,17β-diol	0.03
Epiandrosterone	0.02
3α-Chloro-5α-androstan-17-one	0 02
Androst-5-ene-3β,17α-diol	0.01
3β-Hydroxyandrost-5-en-17-amine	0 01
Androst-4,6-diene-3,17-dione	0 01

[a] Data of Tschopp (1935) and Ruzicka *et al.* (1934), from Selye (1943).

TABLE VIII

RELATIVE POTENCY OF ANDROGENS BY A CAPON'S COMB INJECTION TEST[a]

Steroid	Relative potency (testosterone = 1)
5α-Androstane-3α,17β-diol	0.6
17β-Hydroxy-5α-androstan-3-one	0.6
Androsterone	0.16
Androst-4-ene-3β,17β-diol	0 14
Androst-4-ene-3,17-dione	0.14
5α-Androstane-3,17-dione	0.09
Androst-5-ene-3β,17β-diol	0.02
Epiandrosterone	0.02
Ethynyltestosterone	0.01
Androst-4-ene-3,6,17-trione	0.01
Pregn-4,16-diene-3,20-dione	0.01

[a] Butenandt and Tscherning (1934) and Selye (1943)

TABLE IX

RELATIVE POTENCY OF ANDROGENS BY A CAPON'S COMB INUNCTION TEST, LOCAL APPLICATION IN OIL[a]

Steroid	Relative potency (testosterone = 1)
Androst-4-ene-3,17-dione	2.5
5α-Androstane-3α,17β-diol	2.0
Androst-4-ene-3β,17β-diol	1.0
Androsterone	0.8
5α-Androstane-3β,17β-diol	0.2
Epiandrosterone	0.1
Pregna-4,16-diene-3,20-dione	0.05
Androst-5,7-diene-3β,17β-diol diacetate	0.03
Androst-3,5-dien-17β-ol	0.02

[a] Data of Fussgänger (1934) and Voss (1937), from Selye (1943).

TABLE X

RELATIVE POTENCY OF ANDROGENS BY A CAPON'S COMB INUNCTION TEST[a]

Steroid	Relative potency (testosterone = 1)
17α-Methyltestosterone	1.0
Androst-4-ene-3,17-dione	1.0
5α-Androstane-3α,17β-diol	0.5
Androsterone	0.5
5α-Androstane-3,11,17-trione	0.2
Adrenosterone	0.1
5α-Androstane-3β,17β-diol	0.1

[a] Dessau (1935, 1937) and Selye (1943).

TABLE XI

RELATIVE POTENCY OF ANDROGENS BY THE METHOD OF EISENBERG AND GORDAN (1950) (GAVAGE)

Steroid	Relative potency (fluoxymesterone = 1)	Reference
16α,17α-Dimethyl-17β-hydroxy-androsta-4,9(11)-dien-3-one	0.17	Fried *et al.* (1962)

TABLE XII

RELATIVE POTENCY OF ANDROGENS BY THE METHOD OF EISENBERG AND GORDAN (1950)
(SUBCUTANEOUS INJECTION)

Steroid	Relative potency (testosterone propionate = 1)	References
17α-Methyl-19-nortestosterone	0.06	Saunders and Drill (1956)
17α-Ethyl-19-nortestosterone	0.06	Saunders and Drill (1956, 1957)
17α-Propyl-19-nortestosterone	0.01	Saunders and Drill (1956)
17α-Vinyl-19-nortestosterone	0.02	Saunders and Drill (1956, 1957)
16,16-Difluoro-17β-hydroxyandrost-4 en-3-one	0.025	Goldkamp (1962)
Androsta-4,6-diene-3β,17β-diol 17-acetate	0.02	Baran (1963)
Androsta-4,6-diene-3β,17β-diol 3,17-diacetate	0.01	
17α-Methylandrosta-4,6-diene-3β,17β-diol	0.05	
17α-Methylandrosta-4,6-diene-3β,17β-diol 3-acetate	0.05	
17α-Methylandrosta-4,6-diene-3β,17β-diol 3-propionate	0	
Testosterone	0.35	Counsell and Klimstra (1962)
Methyltestosterone	0.24	
17β-Hydroxy-5α-androstan-3-one	0.50	
2-Chloro-17β-hydroxy-5α-androstan-3-one acetate	0.10	
2-Bromo-17β-hydroxy-5α-androstan-3-one	0.10	
17α-Methyl-2-chloro-17β-hydroxy-5α-androstan-3-one	0.10	
17α-Methyl-2-bromo-17β-hydroxy-5α-androstan-3-one	0.50	
17α-Methyl-2-chloro-5α-androstane-3β,17β-diol	0.10	
17α-Methyl-2-chloro-5α-androst-1-en-3β,17β-diol-3-acetate	0.05	

TABLE XII—*continued*

Steroid	Relative potency (testosterone propionate = 1)	References
Androsta-1,4-diene-3,17-dione	< 0.05	Drill and Riegel (1958)
2-Hydroxyandrosta-1,4-diene-3,17-dione	< 0.01	
4-Hydroxytestosterone	0.01	
2α-Methyltestosterone	0.10	
4-Methyltestosterone	0.10	
6α-Methyltestosterone acetate	0.10	
6β-Methyltestosterone acetate	0.25–0.40	
19-Nortestosterone	0.06	
17α-Methyl-19-nortestosterone	0.06	
17α-Ethyl-19-nortestosterone	0.06	
17α-Propyl-19-nortestosterone	< 0.01	
17α-Vinyl-19-nortestosterone	0.02	
17α-Ethynyl-19-nortestosterone	0.01–0.05	
17α-1-Methallyl-19-nortestosterone	0.01	
17α-2-Methallyl-19-nortestosterone	0.01	
17β-Hydroxyandrost-5(10)-en-3-one	0.10–0.20	
4-Methyl-19-nortestosterone	0.05	
4-Methyl-19-nortestosterone	0.10	
17α-Ethyl-5α-19-norandrostan-3-one	0.02–0.05	
17α-Ethyl-19-norandrost-5(10)-en-3-one	0.01	
17α-Methyl-19-norandrost-5(10)-en-3-one	0.05	
4-Methyl-17α,10-dimethyl-19-nortestosterone	0.02	
11β-Hydroxy-17α,10-dimethyl-19-nortestosterone	0.10	
19-Norandrost-5-ene-3β,17β-diol	0.03	
19-Norandrost-4-ene-3β,17β-diol	0.05	
17α-Ethyl-19-norandrost-4-ene-3β,17β-diol	0.25	
17β-Hydroxy-5α-androst-1-en-3-one	0.50	Counsell and Klimstra (1962)
2-Chloro-17β-acetoxy-5α-androst-1-en-3-one	0.10	
2-Bromo-17β-hydroxy-5α-androst-1-en-3-one	0.10	
2-Chloro-17α-methyl-17β-hydroxy-5α-androst-1-en-3-one	0.10	
2-Bromo-17α-methyl-17β-hydroxy-5α-androst-1-en-3-one	0.50	

continued

TABLE XII—*continued*

Steroid	Relative potency (testosterone propionate = 1)	References
2-Chloro-17α-methyl-5α-androst-1-ene-3β,17β-diol	0.10	
3-Chloro-17α-methyl-5α-androst-1-ene-3β,17β-diol 3-acetate	0.05	
Testosterone	0.35	
Methyltestosterone	0.24	
17α-Methylandrost-5-ene-3β,17β-diol	0.02	Saunders and Drill (1957)
17β-Hydroxy-5α-androstan-3-one	0.13	
Testosterone	0.35	Drill (1960)
17β-Hydroxy-5α-androstan-3-one	0.13	
Methyltestosterone	0.24	
Dehydroepiandrostrone	0.08	
Androst-5-ene-3β,17β-diol	0.14	
17α-Vinyltestosterone	0.02	
19-Nortestosterone	0.06	
17-Ethyl-19-nortestosterone	0.06	
17-Ethynyl-19-nortestosterone	0.02	
17-Ethynyltestosterone	0.02	
17α-Propyltestosterone	< 0.01	
17α-Methyl-19-norprogesterone	0.06	
2-Oxa-17α-methyltestosterone	0.2[a]	Pappo and Jung (1962)
Testosterone	0.35	Saunders and Drill (1957)
Methyltestosterone	0.24	
Methylandrostenediol	0.05	
17β-Hydroxy-5α-androstan-3-one	0.30	
17-Ethyl-19-nortestosterone	0.07	
19-Nortestosterone	0.06	Saunders *et al.* (1957)
17α-Methyl-19-nortestosterone	0.06	
17α-Ethyl-19-nortestosterone	0.06	
11β-Hydroxy-17α-ethyl-19-nortestosterone	< 0.02	
17α-Vinyl-19-nortestosterone	0.02	
17α-Propyl-19-nortestosterone	< 0.05	
17α-Propyl-4,5-dihydro-19-nortestosterone	< 0.02	
17α-Allyl-19-nortestosterone	0.02	
17α-Butyl-19-nortestosterone	< 0.02	
17α-Butenyl-19-nortestosterone	0.02	

[a] Methyltestosterone = 1.

TABLE XIII

RELATIVE POTENCY OF ANDROGENS IN A PREPUTIAL GLAND TEST IN HYPOPHYSECTOMIZED RATS BY INJECTION[a]

Compounds	Relative potency (testosterone = 1)
5α-Androstan-17β-ol	0.4
3β-Fluoro-5α-androstan-17β-ol	0.3
3β-Fluoro-5α-androstan-17-one	< 0 05

[a] Data of Sydnor (1958)

TABLE XIV

RELATIVE POTENCY OF ANDROGENS BY A SINGLE INJECTION TEST IN THE CASTRATE RAT (METHOD OF MATHIESON AND HAYS, 1945)[a]

Compound	Relative potency (testosterone propionate = 1)
Fluoxymesterone	1.0

[a] Clegg and Farley (1962).

TABLE XV

THE RELATIVE POTENCY OF ANDROGENS IN A BONE MATURATION TEST IN MICE[a]

Steroid	Relative potency (testosterone = 1) (confidence limits, $P = 0.95$)
Dehydroepiandrosterone	0.42 (0.20–0.84)
11β-Hydroxyandrost-4-ene-3,17-dione	2
Dehydroepiandrosterone sulfate	1

[a] Howard (1962).

TABLE XVI

RELATIVE POTENCY OF ANDROGENS BY A SEMINAL VESICLE TEST BY INJECTION IN THE IMMATURE RAT[a]

Steroid	Relative potency (testosterone = 1)
Testosterone acetate	4.0
17β-Hydroxy-5α-androstan-3-one	2.0
17β-Acetoxyandrost-5-en-3-one	2.0
17α-Ethyl-17β-hydroxyandrost-5-en-3-one	0.5
5α-Androstane-3α,17β-diol	0.33
Androst-4-ene-3,17-dione	0.14
Androsterone	0.1
Androst-5-ene-3,17-dione	0.06
17α-Methylandrost-5-ene-3β,17β-diol	0.05
Dehydroepiandrosterone	0.03
Epiandrosterone	0.03

[a] Butenandt and Hanisch (1935), Tscherning (1936), and Selye (1943).

TABLE XVII

ANDROGENS DETECTED BY MEANS OF A RAT SEMINAL VESICLE TEST[a]

17β-Methyl-17β-hydroxy-5α-androstan-3-one
Testosterone propionate
17β-Methylandrost-5-ene-3β,17β-diol
Methyltestosterone
Testosterone
17β-Methyl-5α-androstane-3β,17β-dio
Androstene-3β,17β-diol
Androst-4-ene-3,17-dione
Ethynyltestosterone
Androst-5-ene-3β,17β-diol dipropionate
Androsterone

[a] Selye and Albert (1942), Clarke *et al.* (1942), and Selye (1943).

TABLE XVIII

ANDROGENS DETECTED BY MEANS OF A RAT PROSTATE TEST[a]

17β-Methyl-17β-hydroxy-5α-androstan-3-one
Testosterone propionate
17β-Methylandrost-5-ene-3β,17β-diol
Methyltestosterone
Testosterone
17β-Methyl-5α-androstane-3β,17β-diol
Androstene-3β,17β-diol
Androst-4-ene-3,17-dione
Ethynyltestosterone
Androst-5-ene-3β,17β-diol dipropionate
Androsterone
Progesterone
Androst-5-ene-3β,17α diol

[a] Selye and Albert (1942), Clarke *et al* (1942), and Selye (1943).

TABLE XIX

RELATIVE ANDROGENS DETECTED BY MEANS OF A PARABIOSIS INJECTION ASSAY USING THE PROSTATE OF THE CASTRATED RAT[a]

Compound	Relative potency (testosterone = 1)
Testosterone propionate	1.1
4-Chloro-17α-methyl-19-nortestosterone	0.1
2α-Methyl-19-nortestosterone	0 2
2α-Fluoro-17α ethynyltestosterone	0 1
2-Benzoyloxymethylene-17α-methyl 17β-hydroxy-5α-androstan-3-one	0.3
2α-Fluorotestosterone	0.3
2α-Methoxy-17α-methyl-17β-hydroxy-5α-androstan-3-one	0.1
16β-Methyl-19-nortestosterone	0.1
6α-Chloro-17α-acetoxypregna-1,4-diene-3,20-dione	0.005
4,9α-Dichloropregn-4-ene-3,11,20-trione	0.05
2-Methoxymethylene-17β-hydroxy-5α-androstan-3-one	0.04
2,2,17α-Trimethyl-17β-hydroxy-5α-19-norandrostan-3-one	0 03
2,2,17-Trimethyl-17β-hydroxy-5α androstan-3-one	0.03

[a] Kincl *et al.* (1961).

TABLE XX

RELATIVE ANDROGENS DETECTED BY MEANS OF A PARABIOSIS INJECTION ASSAY USING THE SEMINAL VESICLES OF THE CASTRATED RAT[a]

Compound	Relative potency (testosterone = 1)
Testosterone propionate	0.8
4-Chloro-17α-methyl-19-nortestosterone	0.18
2α-Methyl-19-nortestosterone	0.2
2α-Fluoro-17α-ethynyltestosterone	0.2
2-Benzoyloxymethylene-17α-methyl-17β-hydroxy-5α-androstan-3-one	0.2
2α-Fluorotestosterone	0.2
2α-Methoxy-17α-methyl-17β-hydroxy-5α-androstan-3-one	0.1
16β-Methyl-19-nortestosterone	0.1
6α-Chloro-17α-acetoxypregna-1,4-diene-3,20-dione	0.005
4,9α-Dichloropregn-4-ene-3,11,20-trione	0.07
2-Methoxymethylene-17β-hydroxy-5α-androstan-3-one	0.06
2,2,17α-Trimethyl-17β-hydroxy-5α-19-norandrostan-3-one	0.05
2,2,17-Trimethyl-17β-hydroxy-5α-androstan-3-one	0.05

[a] Kincl *et al.* (1961).

TABLE XXI

RELATIVE POTENCY OF ANDROGENS ADMINISTERED BY INJECTION AND DETERMINED BY MEANS OF A SEMINAL VESICLE TEST IN THE CASTRATED RAT[a]

Steroid	Relative potency (95% confidence limits)	Standard	Reference
19-Nortestosterone	0.10	Testosterone = 1	Segaloff (1963)
7α-Methyltestosterone	1.0^{+}		
7α-Methyl-19-nortestosterone	6.0		
19-Nortestosterone acetate	1.0		
7α-Methyltestosterone acetate	5.0		
7α-Methyl-19-nortestosterone acetate	3.0		
Androst-4-ene-3,17-dione	0.2		
19-Norandrost-4-ene-3,17-dione	0.2		

[a] Hershberger *et al.* (1953).

TABLE XXI—*continued*

Steroid	Relative potency (95% confidence limits)	Standard	Reference
7α-Methyl-19-norandrost-4-ene-3,17-dione	0.2		
Methyltestosterone	1.0		
17α-Methyl-19-nortestosterone	0.25		
7α,17α-Dimethyltestosterone	4.0 (Low dose)		
7α,17α-Dimethyl-19-nortestosterone	25		
2α,3α-Difluorocyclopropan-5α-androstan-17β-ol acetate	0.44	Testosterone = 1	Dorfman and Kincl (1963)
2β,3β-Difluorocyclopropan-5α-androstan-17β-ol acetate	0.42		
Ro 2–7239 (dextro, racemic)	Active		Boris (1962)
Ro 2–7239 (levo)	Inactive		
Ro 2–7239 (racemic)	Active		Dorfman and Stevens (1960)
Methyltestosterone dimethylhydrazine	—	Testosterone propionate = 1	McKinney and Payne (1961)
17α-Methylandrost-5-ene-3β,17-diol	0 05	Testosterone propionate = 1	Hershberger *et al.* (1953) Barnes *et al.* (1954a)
19-Nortestosterone	0 1	Testosterone propionate = 1	Hershberger *et al.* (1953)
19-Nortestosterone	0 07	Testosterone propionate = 1	Barnes *et al.* (1954b)
19-Nortestosterone propionate	0 41		
19-Nortestosterone cyclopentylpropionate	0.21		
19-Nortestosterone trimethylacetate	0.02		
19-Nortestosterone benzoate	Ca 0.03	Testosterone propionate = 1	Lerner *et al.* (1959)

continued

TABLE XXI—*continued*

Steroid	Relative potency (95% confidence limits)	Standard	Reference
Methylandrostenol	1.16	Methyltestosterone = 1	Overbeek *et al.* (1962)
17α-Methylandrosta-3β,17β-diol	0.68		
Methylandrostanol	1.00		
17β-Hydroxyandrosta-1,4-dien-3-one	0.45		
Ethylestrenolone	0.33		
Ethylestrenol	0.22		
Methylandrostenediol	0.02	Testosterone propionate = 1	Barnes *et al.* (1954a)
19-Nortestosterone cyclopentylpropionate	0.15		
17β-Hydroxy-5α-androstan-3-one	0.13		
19-Nortestosterone cyclopentylpropionate	0.18		
Methylandrostenediol	0.2	Testosterone propionate = 1	Sala and Baldratti (1957)
19-Nortestosterone cyclopentylpropionate	0.25		
19-Nor-17α-ethyltestosterone	0.1		
4-Chlorotestosterone acetate	0.11		
4-Chlorotestosterone propionate	0.12		
4-Chloro-11β-hydroxytestosterone acetate	0.1		
4-Hydroxytestosterone	0.05		
4-Chloro-19-nortestosterone acetate	0.1		
4-Chloro-19-nortestosterone cyclopentyl propionate	0.05		
4-Hydroxy-19-nortestosterone acetate	0.2		
4-Chloro-17α-methyl-19-nortestosterone	0.1		
2,17α-Dimethyl-5α-androst-2-en-17β-ol	0.87 (0.73–1.06)	Testosterone = 1	Dorfman and Kincl (1963)
2,17α-Dimethyldihydrotestosterone azine	< 0.12		

TABLE XXI—*continued*

Steroid	Relative potency (95% confidence limits)	Standard	Reference
2-Hydroxymethyl-17α-methyl-5α-androst-2-en-17β-ol	0.62 (0.55–0.71)		
2α-Hydroxymethyldihydrotestosterone	0.50 (0.44–0.56)		
2-Cyano-5α-androst-1-en-17β-ol caproate	1.70 (1.48–1.92)		
17α-Ethyl-19-nortestosterone	1.18 (0.97–1.44)		
2-Hydroxymethylene-17α-methyldihydrotestosterone	0.64 (0.53–0.77)		
Δ^1-Dehydro-17α-methyltestosterone	0.50 (0.40–0.60)		
17β-Hydroxy-17α-methylandrostano-(3,2–C)-pyrazole	1.69 (1.39–2.03)		
17α-Methyltestosterone	0.94		
17α-Methyl-19-nortestosterone	0.25 (0.22–0.28)		
17α-Methyldihydrotestosterone	0.78 (0.66–0:92)		
2-Formyl-17α-methyl-5α-androst-2-en-17β-ol	0.50		
2-Methyl-5α-androst-2-en-17β-ol	2.40 (1.78–3.23)		
2-Methyl-5α-androst-2-en-17β-ol-acetate	0.28 (0.23–0.36)		
2-Methylene-17α-methylandrostan-17β-ol	0.40 (0.31–0.52)		
17α-Methyl-5α-androst-2-en-17β-ol	0.88 (0.68–1.03)		
2-Difluoromethyl-5α-androst-2-en-17β-ol-acetate	0.08 (0.06–0.10)		
4-Hydroxy-17α-methyltestosterone	0.42 (0.32–0.54)		
4-Chloro-17α-methyltestosterone	0.26 (0.21–0.32)		
Dihydrotestosterone-3-isonicotinyl hydrazone	0.96 (0.68–1.33)		
9α-Fluoro-11β-hydroxy-17α-methyltestosterone	1.88 (1.56–2.24)		
7α-Methyl-19-nortestosterone acetate	6.5	Testosterone propionate = 1	Campbell *et al.* (1963)

continued

TABLE XXI—*continued*

Steroid	Relative potency (95% confidence limits)	Standard	Reference
17β-Hydroxyandrosta-4,14-dien-3-one	0.08 (0.06–0.12)	Testosterone = 1	Segaloff and Gabbard (1963)
17β-Hydroxy-5α-androstan-3-one	0.66 (0.49–0.89)		
17β-Hydroxy-5α-androst-14-en-3-one	0.15 (0.12–0.19)		
2-Nitrilo-5α-androst-2-en-17β-ol	2.0 (1.3–2.9)	Testosterone = 1	Kincl and Dorfman (1964)
2-Hydroxymethyl-5α-androst-2-en-17β-ol	0.50[b]		
2-Methyl-5α-androst-1-en-17β-ol acetate	0.24 (0.19–0.29)		
2-Formyl-5α-androst-2-en-17β-ol	0.1[b]		
2-Hydroxyethyl-5α-androst-2-en-17β-ol	0.05[b]		
2-Methylene-5α-androstan-17β-ol acetate	0.09 (0.08–0.1)		
2α,3α-Difluoromethylene-5α-androstan-17β-ol acetate	0.44 (0.38–0.51)		
2α,3α-Difluromethylene-17α-methyl-5α-androstan-17β-ol	0.11 (0.10–0 13)		
2β,3β-Difluoromethylene-5α-androstan-17β-ol acetate	0.42 (0.33–0.53)		
5α-Androst-1-en-17β-ol acetate	0.65 (0.60–0.70)		
	0.72 (0.64–0.85)		
5α-Androst-3-en-17β-ol acetate	0.40 (0.38–0.43)		
5α-Androsta-1,3-dien-17β-ol acetate	0.50 (0.47–0.53)		
	0.36 (0.34–0.39)		
2-Fluoromethyl-5α-androst-2-en-17β-ol acetate	0.48 (0.43–0.54)		
	0.45 (0.40–0.50)		
Testosterone acetate	0.93 (0.87–0.99)	Testosterone = 1	Kincl and Dorfman (1964)
Testosterone 17-methylether	0.23 (0.20–0.26)		

TABLE XXI—*continued*

Steroid	Relative potency (95% confidence limits)	Standard	Reference
Testosterone 17-dichloroacetate	7.69 (6.92–8.54)		
Testosterone 17-fluorochloroacetate	3.61 (3.36–3.90)		
2α-Methyl-17β-hydroxy-5α-androstan-3-one	0.26 (0.24–0.28)		
2α-Methyl-17β-hydroxy-5α androstan-3-one propionate	0.36 (0.34–0.39)		
2-Methyl-17β-acetoxy-5α-androst-1-en-3-one	1.36 (1.14–1.50)		
	1.07 (0.95–1.20)		
6α-Fluorotestosterone	1.28 (1.05–1.55)		
6-Chloro-17β-acetoxyandrosta-4,6-dien-3-one	0.42 (0.34–0.51)		
19-Methylene-17β-hydroxy-androst-4-en-3-one	0.89 (0.84–0.95)		
19-Methyl-17β-hydroxy-androst-4-en-3-one	0.03 (0.02–0.04)		
17α-Hydroxyandrost-4-en-3-one	0.03 (0.02–0.04)		

[b] Graphic estimate.

TABLE XXII

RELATIVE POTENCY OF ANDROGENS ADMINISTERED BY INJECTION AND DETERMINED BY A PROSTATE TEST IN THE CASTRATED RAT[a]

Steroid	Relative potency (95% confidence limits)	Standard	References
17β-Hydroxyandrosta-4,14-dien-3-one	0.31 (0.20–0.49)	Testosterone = 1	Segaloff and Gabbard (1963)
17β-Hydroxy-5α-androstan-3-one	0.19 (0.13–0.28)		

[a] Hershberger *et al.* (1953).

continued

TABLE XXII—*continued*

Steroid	Relative potency (95% confidence limits)	Standard	References
17β-Hydroxy-5α-androsten-14-en-3-one	0.70 (0.44–1.03)		
17β-Hydroxy-5β-androstan-3-one	0.04 (0.03–0.05)		
17β-Hydroxy-5β-androst-14-en-3-one	0.09 (0.06–0.13)		
17β-Hydroxy-5α-androstan-3-one	0.91 (0.7–1.18)	Testosterone = 1	Segaloff and Gabbard (1962)
5α-Androstane-3,17-dione	1.94 (1.51–2.50)		
17β-Hydroxy-5β-androstan-3-one	1.0 (0.78–1.35)		
Androst-4-ene-3,17-dione	1.17 (0.84–1.61)		
5α-Androstan-17β-ol	0.02 (0.017–0.029)		
5α-Androst-2-en-17β-ol	0.18 (0.14–0.22)		
Androst-4-en-17β-ol	0.06 (0.05–0.08)		
19-Nortestosterone	0.40	Testosterone = 1	Segaloff (1963)
7α-Methyl-19-nortestosterone	1.2		
7α-Methyl-19-nortestosterone	8.0		
19-Nortestosterone acetate	1.0		
7α-Methyltestosterone acetate	1.0 (low doses) 3.0 (high doses)		
17α-Methyl-19-nortestosterone acetate	10^{+}		
Androst-4-ene-3,17-dione	2.0		
19-Norandrost-4-ene-3,17-dione	0.10		
7α-Methylandrost-4-ene-3,17-dione	0.10		
7α-Methyl-19-norandrost-4-ene-3,17-dione	0.17		
Methyltestosterone	2.0		

TABLE XXII—*continued*

Steroid	Relative potency (95% confidence limits)	Standard	References
17α-Methyl-19-nortestosterone	0.7		
7α,17α-Dimethyltestosterone	2.0 (low dose)		
7α,17α-Dimethyl-19-nortestosterone	20		
2α,3α-Difluorocyclopropan-5α-androstan-17β-ol acetate	0.27	Testosterone = 1	Dorfman and Kincl (1963)
2β,3β-Difluorocyclopropan-5α-androstan-17β-ol acetate	0.34		
17β-Hydroxy-5α-androstano-[3,2-*d*]-2′,6′-diamino-pyrimidine	0.03–0 1	Testosterone propionate = 1	Smith *et al.* (1963)
17α-Methyl-17β-hydroxy-5α-androstano-[3,2-*d*]-2′,6′-diaminopyrimidine	0.6		
Ro 2–7239 (dextro, racemic)	Active		Boris (1962)
Ro 2–7239 (levo)	Inactive		
Ro 2–7239 (racemic)	Active		Dorfman and Stevens (1960)
Testosterone cyclopentyl enol ether	Active		Mauli *et al.* (1960)
Methyltestosterone cyclopentyl enol ether	Active		
19-Nortestosterone benzoate	ca. 3	Testosterone propionate = 1	Lerner *et al.* (1959)
19-Nortestosterone	0.3	Testosterone propionate = 1	Wilds and Nelson (1953)
19-Norandrost-4-ene-3,17-dione	0.2		
17β-Hydroxyandrost-5(10)-en-3-one	0.07		

continued

TABLE XXII—*continued*

Steroid	Relative potency (95% confidence limits)	Standard	References
18-Nor-*D*-homo-5α-androstane-3,17α-dione	ca. 0.1	Testosterone = 1	Johnson *et al.* (1953)
7α-Methylthiotestosterone acetate	1.4 (1.2–1.7)	17α-Ethyl-19-nortestosterone = 1	Schaub and Weiss (1961)
7α-Acetylthiotestosterone acetate	0.4 (0.2–1.0)		
7α-Mercaptotestosterone acetate	1.1 (0.7–1.8)		
Androstanazole	0.03	Testosterone propionate = 1	Arnold *et al.* (1959)
Testosterone dimethylhydrazone	0.05	Testosterone propionate = 1	McKinney and Payne (1961)
Methyltestosterone dimethylhydrazone	0.17		
Methylandrostenediol	0.2	Testosterone propionate = 1	Sala and Baldratti (1957)
19-Norcyclopentyl propionate	0.2		
4-Fluorotestosterone acetate	0.04		
4-Chlorotestosterone acetate	0.2		
4-Bromotestosterone	0.06		
4-Chloro-11β-hydroxytestosterone acetate	0.1		
4-Hydroxytestosterone acetate	0.06		
4-Chloro-19-nortestosterone acetate	0.16		
4-Hydroxy-19-nortestosterone acetate	0.13		
4-Chloro-17α-methyl-19-nortestosterone	0.06		
2,17α-Dimethyl-(5α)-Δ^2-androstan-17β-ol	0.64 (0.52–0.79)	Testosterone = 1	Dorfman and Kincl (1963)
2,17α-Dimethyldihydrotestosterone azine	< 0.06		
2-Hydroxymethyl-17α-methyl-Δ^2-(5α)-androsten-17β-ol	0.45 (0.38–0.54)		

TABLE XXII—*continued*

Steroid	Relative potency (95% confidence limits)	Standard	References
2α-Hydroxymethyldihydro-testosterone	0.35 (0.28–0.44)		
2-Cyano-Δ^2-(5α)-androsten-17β-ol caproate	1.25 (0.92–1.51)		
17α-Ethyl-19-nortestosterone	0.94 (0.71–1.25)		
2-Hydroxymethylene-17α-methyldihydrotestosterone	1.0 (0.75–1.33)		
Δ^1-Dehydro-17α-methyltestosterone	0.55 (0.41–0.73)		
17β-Hydroxy-17α-methylandrostano-(3,2-*C*)-pyrazole	1.36 (1.02–1.81)		
17α-Methyltestosterone	1.01 (0.78–1.32)		
	1.05 (0.76–1.46)		
17α-Methyl-19-nortestosterone	0.25 (0.20–0.30)		
17α-Methyldihydro-testosterone	2.54 (1.94–3.33)		
2-Formyl-17α-methyl-5α-androsten-17β-ol	0.18		
2-Methyl-5α-androst-2-en-17β-ol	1.27 (0.84–1.92)		
2-Methyl-5α-androst-2-en-17β-ol acetate	0.45 (0.34–0.60)		
2-Methylene-17α-methylandrostan-17β-ol	0.37 (0.30–0.44)		
17α-Methyl-5α-androst-2-en-17β-ol	0.70 (0.56–0.84)		
2-Difluoromethyl-5α-androst-2-en-17β-ol acetate	0.044 (0.031–0.063)		
4-Hydroxy-17α-methyltestosterone	0.61 (0.43–0.87)		Dorfman and Kincl (1963)
4-Chloro-17α-methyltestosterone	0.27 (0.192–0.38)		
Dihydrotestosterone-3-isonicotinyl hydrazone	0.72 (0.35–1.30)		
9α-Fluoro-11β-hydroxy-17α-methyltestosterone	0.75 (0.58–0.98)		

continued

TABLE XXII—*continued*

Steroid	Relative potency (95% confidence limits)	Standard	References
2-Nitrilo-5α-androst-2-en-17β-ol	0.94 (0.68–1.39)	Testosterone = 1	Kincl and Dorfman (1964)
2-Hydroxymethyl-5α-androst-2-en-17β-ol	0.66[b]		
2-Methyl-5α-androst-1-en-17β-ol acetate	0.28 (0.26–0.31)		
2-Formyl-5α-androst-2-en-17β-ol	0.1[b]		
2-Hydroxyethyl-5α-androst-2-en-17β-ol	0.05[b]		
2-Methylene-5α-androstan-17β-ol acetate	0.04 (0.03–0.06)		
2α,3α-Difluoromethylene-5α-androstan-17β-ol acetate	0.27 (0.21–0.35)		
2α,3α-Difluoromethylene-17α-methyl-5α-androstan-17β-ol	0.05 (0.04–0.05)		
2β,3β-Difluoromethylene-5α-androstan-17β-ol acetate	0.34 (0.24–0.49)		
5α-Androst-1-en-17β-ol acetate	0.64 (0.54–0.76)		
	0.89 (0.74–1.07)		
5α-Androst-3-en-17β-ol acetate	0.26 (0.24–0.28)		
5α-Androsta-1,3-dien-17β-ol acetate	0.37 (0.34–0.40)		
	0.30 (0.28–0.33)		
2-Fluoromethyl-5α-androst-2-en-17β-ol acetate	0.52 (0.47–0.57)		
	0.41 (0.36–0.46)		
Testosterone acetate	0.97 (0.92–1.02)		
Testosterone 17-methylether	0.34 (0.31–0.39)		
Testosterone 17-dichloro acetate	10.33 (8.68–12.29)		
Testosterone 17-fluorochloro acetate	2.33 (2.06–2.63)		

[b] Graphic estimate.

TABLE XXII—*continued*

Steroid	Relative potency (95% confidence limits)	Standard	References
2α-Methyl-17β-hydroxy-5α-androstan-3-one	0.24 (0.22–0.26)		
2α-Methyl-17β-hydroxy-5α-androstan-3-one-propionate	0.49 (0.43–0.56)		
2-Methyl-17β-acetoxy-5α-androst-1-en-3-one	1.20 (1.08–1.33)		
	1.44 (1.22–1.69)		
6α-Fluorotestosterone	1.23 (1.02–1.53)		
6-Chloro-17β-acetoxy-androsta-4,6-dien-3-one	0.14 (0.09–0.22)		
19-Methylene-17β-hydroxy-androst-4-en-3-one	0.75 (0.67–0.83)		
19-Methyl-17β-hydroxy-androst-4-en-3-one	0.05 (0.04–0.06)		
17α-Hydroxyandrost-4-en-3-one	0.08 (0.07–0.09)		

TABLE XXIII

RELATIVE POTENCY OF ANDROGENS ADMINISTERED BY GAVAGE AND DETERMINED BY A SEMINAL VESICLE TEST IN THE CASTRATED RAT[a]

Steroid	Relative potency (95% confidence limits)	Standard	References
11β-Hydroxymethyltestosterone	0.9 (0.7–1.1)	Methyltestosterone = 1	Lyster *et al.* (1956)
Fluoxymesterone	95 (9–10)		
11-Ketomethyltestosterone	0.65 (0.6–0.7)		
9α-Fluoro-11-ketomethyl-testosterone	8.5 (7.0–10.0)		

continued

TABLE XXIII—*continued*

Steroid	Relative potency (95% confidence limits)	Standard	References
17α-Methyl-11,17α-dihydroxy-19-norandrost-4-en-3-one	2.6	Methyltestosterone = 1	Magerlein and Hogg (1958)
17α-Ethyl-11β,17α-dihydroxy-19-norandrost-4-en-3-one	0.8		
7α,17α-Dimethyltestosterone	0.33	Fluoxymesterone = 1	Stucki *et al.* (1962)
Testosterone	0.50	Methyltestosterone = 1	Dorfman and Kincl (1963)
17α-Methyl-17β-hydroxy-androsta-1,4-dien-3-one	0.12 (0.09–0.17)		
2-Hydroxymethylene-17α-methyl-17β-hydroxy-5α-androstan-3-one	0.44		
2,17α-Dimethyl-5α-androst-2-en-17β-ol	3.20 (2.82–3.58)		
Fluoxymesterone	7.60		
4-Chloro-17α-methyltestosterone	ca. 0.10		
2-Hydroxymethyl-17α-methyl-5α-androst-2-en-17β-ol	0.44 (0.29–0.69)		
2-Methylene-(*N*,*N*-diethylamino)-17α-methyl-17β-hydroxy-5α-androstan-3-one	0.40		
7α-Methyl-19-nortestosterone	2.5	Methyltestosterone = 1	Campbell *et al.* (1963).
7α-Methyl-19-nortestosterone acetate	5.4		
7α,17α-Methyl-19-nortestosterone	18		
5α-Androstan-17β-ol-3-one 17-(1′-ethoxy)cyclopentyl ether	2.7	Methyltestosterone = 8	Ercoli *et al.* (1962)
5α-Androstan-17β-ol-3-one 17-(cyclopent-1′-enyl) ether	0.7		
Testosterone 17-(1′-ethoxy)-cyclopentyl ether	1.9		
Testosterone 17-(cyclopent-1′-enyl) ether	1.3		

TABLE XXIII—*continued*

Steroid	Relative potency (95% confidence limits)	Standard	References
Testosterone 17-(1′-ethoxy)-cyclohexyl ether	1		
Androsta-1,4-diene-17β-ol-3-one 17-(cyclopent-1′-enyl) ether	0.2		
5α-Androst-1-ene-17β-ol-3-one 17-(1′-ethoxy)-cyclopentyl ether	2.5		
5α-Androst-1-ene-17β-ol-3-one 17-(cyclopent-1′-enyl) ether	2.4		
Androst-4-ene-3β,17β-diol 17-(1′-ethoxy)cyclopentyl ether	2.3		
Androst-4-ene-3α,17α-diol 17-(cyclopent-1′-enyl) ether	1.5		
5α-Androstane-3α,17β-diol 17-(cyclopent-1′-enyl) ether	1.1		
5α-Androstane-3α,17β-diol 3-acetate 17-(1′-ethoxy-1′-methyl)ethyl ether	1.2		
19-Nortestosterone 17-(cyclopent-1′-enyl) ether	0.4		
Estra-5(10)-en-17β-ol 17-(cyclopent-1′-enyl) ether	0.4		
19-Nortestosterone	1.0	Methyltestosterone = 1	Segaloff (1963)
Testosterone	0.25		
7α-Methyltestosterone	< 0.20		
7α-Methyl-19-nortestosterone	4.0		
Testosterone acetate	< 0.2		
19-Nortestosterone acetate	1.0		
7α-Methyltestosterone 17-acetate	< 0.2		
7α-Methyl-19-nortestosterone acetate	0.5		
Androst-4-ene-3,17-dione	< 0.10		
19-Norandrost-4-ene-3,17-dione	0.05		
7α-Methylandrost-4-ene-3,17-dione	< 0.10		

continued

TABLE XXIII—*continued*

Steroid	Relative potency (95% confidence limits)	Standard	References
7α-Methyl-19-norandrost-4-ene-3,17-dione	1.0		
17α-Methyl-19-nortestosterone	0.7		
7,17α-Dimethyltestosterone	2.0		
7α,17α-Dimethyl-19-nortestosterone	ca. 50		
2-Formyl-17α-methyl-17β-hydroxy-5α-androst-1-en-3-one	0.53 (0.36–1.0)	Methyltestosterone = 1	Kincl and Dorfman (1964)
2-Cyano-17α-methyl-5α-androst-2-en-17β-ol	0.73 (0.54–1.09)		
2-Nitrilo-17α-methyl-5α-androst-2-en-17β-ol acetate	0.17 (0.13–0.22)		
2-Formyl-17α-methyl-5α-androst-2-en-17β-ol	0.25[b]		
2-Carboxy-17α-methyl-5α-androst-2-en-17β-ol	0.48 (0.40–0.57)		
2-Methylene-17α-methyl-5α-androstan-17β-ol	1.46 (1.31–1.66)		
3-Methylene-17α-methyl-5α-androstan-17β-ol	0.32 (0.25–0.41)		
17α-Methyl-5α-androst-2-en-17β-ol	2.18 (2.11–2.38)		
2α,3α-Difluorocyclopropane-17α-methyl-5α-androst-2-en-17β-ol	0.65 (0.52–0.70)		

[a] Hershberger *et al.* (1953).
[b] Graphic estimate.

TABLE XXIV

RELATIVE POTENCY OF ANDROGENS ADMINISTERED BY GAVAGE AND DETERMINED BY A PROSTATE TEST IN THE CASTRATED RAT[a]

Steroid	Relative potency (95% confidence limits)	Standard	References
17β-Hydroxyandrosta-4,14-dien-3-one	1.18 (0.83–1.70)	Testosterone = 1	Segaloff and Gabbard (1963)
17β-Hydroxy-5α-androstan-3-one	1.12 (0.78–1.62)		
17β-Hydroxy-5α-androst-14-en-3-one	1.73 (1.04–2.86)		
17β-Hydroxy-5β-androstan-3-one	0.13 (0.078–0.202)		
17β-Hydroxy-5α-androstan-3-one	2.06 (1.17–3.58)	Testosterone = 1	Segaloff and Gabbard (1962)
17β-Hydroxy-5β-androstan-3-one	0.12 (0.05–0.022)		
5α-Androstane-3,17-dione	3.95 (2.37–6.60)		
Androst-4-ene-3,17-dione	1.93 (1.12–3.36)		
5α-Androstan-17β-ol	0.16 (0.096–0.27)		
5α-Androst-2-en-17β-ol	2.46 (1.47–4.10)		
Androst-4-en-17β-ol	13.0 (7.80–21.70)		
Testosterone	0.07	Methyltestosterone = 1	Segaloff (1963)
19-Nortestosterone	0.07		
7α-Methyltestosterone	0.07		
7α-Methyl-19-nortestosterone	1.0		
Testosterone acetate	0.03		
19-Nortestosterone acetate	0.01		
7α-Methyltestosterone acetate	0.02		
7α-Methyl-19-nortestosterone acetate	1.0		
Androst-4-ene-3,17-dione	0.04		

[a] Hershberger *et al.* (1953).

continued

TABLE XXIV—*continued*

Steroid	Relative potency (95% confidence limits)	Standard	References
19-Norandrost-4-ene-3,17-dione	0.01		
7α-Methylandrost-4-ene-3,17-dione	0.01		
7α-Methyl-19-norandrost-4-ene-3,17-dione	0.2		
17α-Methyl-19-nortestosterone	1.0		
7α,17α-Dimethyltestosterone	1.0		
7α,17α-Dimethyl-19-nortestosterone	18		
7α-Methylthiotestosterone	0.4 (0.2–1.0)	17α-Ethyl-19-nortestosterone = 1	Schaub and Weiss (1961)
Androstanazole	0.25	Methyltestosterone = 1	Arnold *et al.* (1959)
Testosterone	0.28	Methyltestosterone = 1	Dorfman and Kincl (1963)
17α-Methyl-17β-hydroxyandrosta-1,4-dien-3-one	0.06		
2-Hydroxymethylene-17α-methyl-17β-hydroxy-5α-androstan-3-one	0.45		
2,17α-Dimethyl-5α-androst-2-en-17β-ol	0.97 (0.70–1.34)		
Fluoxymesterone	1.18		
4-Chloro-17α-methyltestosterone	0.15 (0.10–0.21)		
2-Hydroxymethyl-17α-methyl-5α-androst-2-en-17β-ol	0.19 (0.14–0.25)		
2-Methylene-(*N*,*N*-diethylamino)-17α-methyl-17β-hydroxy-5α-androstan-3-one	0.40		
2-Formyl-17α-methyl-17β-hydroxy-5α-androst-1-en-3-one	0.60[a]	Methyltestosterone = 1	Dorfman and Kincl (1964)
2-Cyano-17α-methyl-5α-androst-2-en-17β-ol	0.20[a]		

[a] Graphic estimate.

TABLE XXIV—*continued*

Steroid	Relative potency (95% confidence limits)	Standard	References
2-Nitrilo-17α-methyl-5α-androst-2-en-17β-ol acetate	0.04 (0.02–0.07)		
2-Formyl-17α-methyl-5α-androst-2-en-17β-ol	0.20[b]		
2-Carboxy-17α-methyl-5α-androst-2-en-17β-ol	0.18 (0.06–0.28)		
2-Methylene-17α-methyl-5α-androstan-17β-ol	0.75 (0.58–0.97)		
3-Methylene-17α-methyl-5α-androstan-17β-ol	0.84 (0.52–1.18)		
17α-Methyl-5α-androst-2-en-17β-ol	1.19 (0.73–1.96)		
2α,3α-Difluorocyclopropane-17α-methyl-5α-androst-2-en-17β-ol	0.32 (0.30–0.35)		

[b] Graphic estimate.

TABLE XXV

COMPOUNDS REPORTED TO BE ANDROGENIC BY UNSPECIFIED TESTS

Compound (relative potency if available)	References
10β-Hydroxy-19-nortestosterone-17-acetate (TP× <0.1)	Drill and Riegel (1958)
11β-Hydroxy-17α-ethyl-19-nortestosterone (TP<0.1 SQ)	Drill and Riegel (1958)
16β-Methyl-19-nortestosterone	Kincl and Garcia (1959)
16-Keto-19-nortestosterone	Kincl and Garcia (1959)
17α-Ethylene-19-nortestosterone	Sandoval *et al.* (1953, 1955)
6α-Methyltestosterone	Campbell *et al.* (1958)
6β-Methyltestosterone	Campbell *et al.* (1958)
6α,17α-Dimethyltestosterone	Campbell *et al.* (1958)
6β,17α-Dimethyltestosterone	Campbell *et al.* (1958)
17aβ-Hydroxy-*D*-homoandrost-4-ene-3,11-dione propionate	Clinton *et al.* (1957)

continued

TABLE XXV—*continued*

Compound (relative potency if available)	References
10β-Hydroxy-19-nortestosterone	Ruelas *et al.* (1958)
5α-Fluoro-17α-ethynyl-10β,17β-dihydroxy-19-nor-5α-androstan-3-one	Ruelas *et al.* (1958)
2ξ-Fluorotestosterone	Nathan *et al.* (1959, 1960)
4,17α-Dimethyl-17β-hydroxy-4-azaandrost-5-en-3-one	Doorenbos and Huang (1961)
4-(β-Hydroxy)-17α-methyl-17β-hydroxy-4-azaandrost-5-en-3-one	Doorenbos and Huang (1961)
17β-Formylandrost-4-en-3-one	Miescher *et al.* (1940)

TABLE XXVI

ANDROGENIC POTENCY OF STEROIDS IN A CASTRATED RAT ASSAY BY THE METHOD OF HERSHBERGER ET AL. (1953) — END POINT NOT SPECIFIED

Compound	Route	Relative potency	Standard	References
2α-Fluorotestosterone	Injection	0.2	Testosterone = 1	Edwards and Ringold (1959)
2α-Fluoro-17β-hydroxy-5α-androstan-3-one	Injection	0 5	Testosterone = 1	Edwards and Ringold (1959)
2α-Fluoromethyl-testosterone	Oral	0.25	Methyltestos-terone = 1	Edwards and Ringold (1959)
2α-Methyl-17β-hydroxy-5α-androstan-3-one propionate	Injection	0 5	Testosterone propionate = 1	Ringold *et al.* (1959)
17α-Methyl-19-nor-androst-5(6)-3β,17β-diol	Oral	0.5	Methyltestos-terone = 1	Iriarte *et al.* (1959)
17α-Ethyl-19-nor-androst-5(6)-ene-3β,17β-diol	Oral	0.2	Methyltestos-terone = 1	Iriarte *et al.* (1959)
17β-Benzoyloxy-4-oxa-5β-androstan-3-one	Injection	Low activity	—	Atwater and Ralls (1960)
17β-Benzoyloxy-5-methoxy-4-oxaandro-stan-3-one	Injection	Low activity	—	Atwater and Ralls (1960)
17β-Benzoyloxy-4-oxa-5α-androstan-3-one	Injection	Low activity	—	Atwater and Ralls (1960)

TABLE XXVI—*continued*

Compound	Route	Relative potency	Standard	References
4-Methyl-19-nortestosterone	Injection	0.05	Testosterone propionate = 1	Atwater (1960)
4-Methyltestosterone	Injection	0.10	Testosterone propionate = 1	Atwater (1960)
17β-Hydroxy-17α-methyl-5α-androst-1-en-3-one	Oral	Low	Methyltestosterone = 1	Mauli *et al.* (1960)
17β-Dihydroxy-5α-androsta-1,4-dien-3-one	Oral	Low	Methyltestosterone = 1	Mauli *et al.* (1960)
9α-Bromo-11β-chloro-17β-hydroxyandrosta-1,4-dien-3-one propionate	Injection	0 04	Testosterone propionate = 1	Robinson *et al.* (1960)
9α-Bromo-11β-fluoro-17β-hydroxyandrosta-1,4-dien-3-one propionate	Injection	0.06	Testosterone propionate = 1	Robinson *et al.* (1960)
9α-Chloro-11β-fluoro-17β-hydroxyandrosta-1,4-dien-3-one propionate	Injection	0.05	Testosterone propionate = 1	Robinson *et al* (1960)
9α-Bromo-11β-fluoro-17α-methyl-17β-hydroxyandrosta-1,4 dien-3-one	Oral	0 4	Methyltestosterone = 1	Robinson *et al.* (1960)
9α-Bromo-11β-chloro-17α-methyl-17β-hydroxyandrosta-1,4-dien-3-one	Oral	0 6	Methyltestosterone = 1	Robinson *et al.* (1960)
9α-Chloro-11β-fluoro-17α-methyl-17β-hydroxyandrosta-1,4-dien-3-one	Oral	0 12	Methyltestosterone = 1	Robinson *et al.* (1960)
2,17α-Dimethyl-5α-androst-2-en-17β-ol	Oral	0.3–0.5	Methyltestosterone = 1	Cross *et al.* (1962)
2-Methylene-5α-androstan-17β-ol	Oral	0.3–0.5	Methyltestosterone = 1	Cross *et al.* (1962)
2-Formyl-5α-androst-2-en-17β-ol	Injection	0.2–0 4	Testosterone propionate = 1	Orr *et al.* (1962)

continued

TABLE XXVI—*continued*

Compound	Route	Relative potency	Standard	References
2-Hydroxymethyl-5α-androst-2-en-17β-ol	Injection	0.2–0.4	Testosterone propionate = 1	Orr *et al.* (1962)
17β-Hydroxy-17α-methylandrost-2-en-2-ol	Oral	0.3–0.4	Methyltestosterone = 1	Orr *et al.* (1962)
Androsta-5,7,9(11)-triene-3β,17β-diol	Injection	0.2	Testosterone = 1	Nes *et al.* (1958)
4-Methyltestosterone	Injection	0.4	Testosterone = 1	Sondheimer and Mazur (1957)
11β-Hydroxymethyl-testosterone	Oral	0.9	Methyltestosterone = 1	Herr *et al.* (1956)
Fluoxymesterone	Oral	9.5	Methyltestosterone = 1	Herr *et al.* (1956)
17β-Hydroxy-9α-fluoro-17α-methylandrost-4-ene-3,11-dione	Oral	8.5	Methyltestosterone = 1	Herr *et al.* (1956)
4-Chlorotestosterone	Injection	0.5	Testosterone = 1	Ringold *et al.* (1956b)
4-Bromotestosterone	Injection	Active	Testosterone = 1	Ringold *et al.* (1956b)
4-Methyltestosterone	Injection	0.4	Testosterone = 1	Ringold and Rosenkranz (1957)
17α-Methylandrost-4-ene-3β,17β-diol	Oral	0.25–0.5	Methyltestosterone = 1	Bernstein *et al.* (1957)
17α-Methylandrost-5-ene-3β,17β-diol	Oral	0.25	Methyltestosterone = 1	Bernstein *et al.* (1957)
17α-Methylandrost-4-ene-3β,17β-diol	Injection (single, prostate)	1	Testosterone propionate = 1	Bernstein *et al.* (1957)
Testosterone	Injection (single, prostate)	0.25	Testosterone propionate = 1	Bernstein *et al.* (1957)
Methyltestosterone	Injection (single, prostate)	0.25	Testosterone propionate = 1	Bernstein *et al.* (1957)
6α-Methyltestosterone	Injection	0.9	Testosterone = 1	Ringold *et al.* (1957)
6β-Methyl-17β-hydroxy-5α-androstan-3-one	Injection	3.9	Testosterone = 1	Ringold *et al.* (1957)

TABLE XXVII

STEROIDS INACTIVE IN A CHICK COMB ANDROGEN ASSAY (ABSOLUTE ETHANOL INUNCTION)

Steroid	Relative potency (testosterone = 1)
6α,11β-Dihydroxyandrost-4-ene-3,17-dione	< 0.02
2α-Hydroxytestosterone	< 0.06
16-(*N*-pyrrolidinemethylene)-7β-hydroxy-5α-androstan-3-one	< 0.005
2-Formyl-5α-androst-2-en-17β-ol	< 0.005
16-(*N*-morpholinemethylene)-3β-hydroxy-5α-androstan-17-one	< 0.003
2-Hydroxymethylene-5α-androst-3-en-17β-ol diacetate	< 0.02
17α-Ethynyl-17β-hydroxy 5α-19-norandrostan-3-one	< 0.02
17β-Hydroxy-17α-methyl-5α-androstano-(3,2-*C*)-pyrazole	< 0.02
2-Hydroxymethyl-5α-androst-2-en-17β-ol	< 0.02
9α-Fluoro-11β-hydroxyandrost-4-ene-3,17-dione	< 0.03
9α-Chloro 11β-hydroxyandrost-4-ene-3,17-dione	< 0.03

TABLE XXVIII

STEROIDS INACTIVE AS ANDROGENS IN CASTRATED RAT TESTS

Steroid	References
Testololactone	Shemano *et al.* (1951)
17β-Methylepitestosterone	Heusser *et al.* (1954)
9α-Fluoro-16α,17α-dimethyl-11β,17β-dihydroxy-androst-4-en-3-one	Fried *et al.* (1962)
17β-Hydroxy-3-oxo-androst-5-en-19-nitrile	Jen and Wolff (1962)
3β,17β-Dihydroxyandrost-5-en-19-nitrile	Jen and Wolff (1962)
3,17-Dioxoandrost-4-en-19-nitrile	Jen and Wolff (1962)
11α-Hydroxymethyltestosterone	Glenn *et al.* (1959)
Androsta-5,7,9(11)-triene-3β,17β-diol < 10% of testosterone	Nes *et al.* (1958)
18,19-Bisnortestosterone (10% of testosterone or less)	Johns (1959)
17α-Methyl-3β,17β-dihydroxyandrost-5-en-7-one	Marshall *et al.* (1957)
17α-Ethyl-3β,17β-dihydroxyandrost-5-en-7-one	Marshall *et al.* (1957)
1ξ-Methyl-19-nortestosterone (less than 0.1 of testosterone)	Ringold *et al.* (1956a)
9α-Chloro-11β-hydroxyandrost-4-ene-3,17-dione	Lenhard and Bernstein (1955)
9α-Fluoro-11β-hydroxyandrost-4-ene-3,17-dione	Lenhard and Bernstein (1955)

continued

TABLE—XXVIII—*continued*

Steroid	References
$9\alpha,11\beta$-Dihydroxylandrost-4-ene-3,17-dione	Lenhard and Bernstein (1955)
19-Nortestosterone dimethylhydrazone	McKinney and Payne (1961)
17α-Ethyl-2-Chloro-17β-hydroxy-5α-androst-2-en-3-one	Counsell and Klimstra (1962)
17α-Ethyl-2-bromo-17β-hydroxy-5α-androst-2-en-3-one	Counsell and Klimstra (1962)
17α-Ethyl-2-bromo-5α-androst-1-en-$3\beta,17\beta$-diol	Counsell and Klimstra (1962)
6-Ketotestosterone	Selye (1943)
Androst-4-ene-3,6,17-trione	Selye (1943)
7-Ketoandrost-5-ene-$3\beta,17\beta$-diol	Selye (1943)
7-Ketoandrosta-3,5-dien-17β-ol	Selye (1943)
Deoxycorticosterone	Selye (1943)
Cholesterol	Selye (1943)
17α-Hydroxy-5α-androstan-3-one	Selye (1943)
Epitestosterone	Selye (1943)
Androst-4-ene-3,17-dione enol benzoate	Selye (1943)
Androst-5-ene-$3\beta,17\alpha$-diol	Selye (1943)

TABLE XXIX

THE COMPARATIVE RELATIVE POTENCIES OF VARIOUS ANDROGENS BY CHICK COMB INUNCTION METHODS EMPLOYING ETHANOL AND ETHER (WITH 1% MINERAL OIL) VEHICLES (TESTOSTERONE = 1)

Androgen	A Dorfman and Dorfman (1962a) Ethanol	B Ofner *et al.* (1962a,b) Ethanol	C Segaloff (1963) Segaloff and Gabbard (1962, 1963) 1% Mineral oil in ether
Androst-4-ene-3,17-dione	1.21	2.6	1.25
17α-Methyltestosterone	1.93	2.31	—
11β-Hydroxyandrost-4-ene-3,17-dione	0.12	0.08	—
17β-Hydroxy-5α-androstan-3-one	2.28	1.07	2.27
5α-Androstane-3,17-dione	0.47	--	2.09
Androsterone	1.0	2.4	—
Dehydroepiandrosterone	0.59	0.66	—
Androst-5-ene-$3\beta,17\beta$-diol	1.05	0.36	—
17β-Hydroxyandrosta-1,4-dien-3-one	0.13	0.30	—
Androst-4-ene-$3\beta,17\beta$-diol	1.17	0.76	—

TABLE XXX

THE COMPARATIVE RELATIVE POTENCY OF ANDROGENS INUNCTED IN ETHANOL TO THE CHICK COMB AND INJECTED IN A TWEEN SUSPENSION (TESTOSTERONE = 1)

Androgen	Dorfman and Dorfman (1962a) Ethanol inunction	Ofner *et al.* (1962a,b) Ethanol inunction	Dorfman and Dorfman (1963) Tween suspension injection
Androst-4-ene-3,17-dione	1.21	2.60	0.17
19-Nortestosterone	–	0.86	0.35
Dehydroepiandrosterone	0.59	0.66	0.65
Androst-4-ene-3β,17β-diol	1.17	0.76	0.49

TABLE XXXI

THE COMPARATIVE RELATIVE POTENCIES OF VARIOUS ANDROGENS USING CORN OIL AND ETHANOL CHICK COMB INUNCTION METHODS (TESTOSTERONE = 1)

Androgen	Dorfman and Dorfman (1962a) Corn oil A	Dorfman and Dorfman (1962a) Ethanol B	Ofner *et al* (1962a, b) Ethanol C
Androst-4-ene-3,17-dione	1 78	1.21	2.60
17α-Methyltestosterone	1.45	1.86	2.31
19-Nortestosterone	0.72	—	0.86
Androsterone	1 60		2.40
Adrenosterone	0 02		0.29
Dehydroepiandrosterone	0.55	0.59	0.66
17α-Methylandrost-5-ene-3β,17β-diol	0 65	—	0.87
17β-Hydroxy-5α-androstan-3-one	1.94	2.28	1.07
Epiandrosterone	0 23	—	0.67
5α-Androstane-3,17-dione	0 18	1.15	1.82
11β-Hydroxyandrost-4-ene-3,17-dione	0.46	—	0.08
Androst-4-ene-3β,17β-diol	—	1 17	0.76
5α-Androst-ene-3,17-dione	2.06	—	1.15
17α-Methyl-17β-hydroxyandrost-4-ene-3,11-dione	0.06	—	0.12
Pregna-4,16-diene-3,20-dione	0.12	—	0.08
17β-Hydroxyandrosta-1,4-diene-3,17-dione	–	0.13	0.30
Androst-5-ene-3β,17β-diol	—	1.05	0.36

TABLE XXXII

THE COMPARATIVE RELATIVE POTENCIES OF VARIOUS ANDROGENS INUNCTED IN CORN OIL TO THE CHICK COMB AND INJECTED IN TWEEN (TESTOSTERONE = 1)

Androgen	Dorfman and Dorfman (1962a) Corn oil A	Dorfman and Dorfman (1963) Tween suspension B
Androst-4-ene-3,17-dione	1.78	0.17
19-Nortestosterone	0.72	0.35
Dehydroepiandrosterone	0.55	0.65

TABLE XXXIII

THE COMPARATIVE RELATIVE POTENCIES OF VARIOUS ANDROGENS INUNCTED IN ETHANOL TO THE CHICK COMB AND INJECTED IN CORN OIL (TESTOSTERONE = 1.0)

Androgen	Dorfman and Dorfman (1962a) Ethanol inunction A	Ofner *et al.* (1962a,b) Ethanol inunction B	Dorfman and Dorfman (1962b) Corn oil injection C
Androst-4-ene-3,17-dione	1.21	2.60	0.21
Testosterone propionate	—	3.80	3.95
17β-Hydroxy-5α-androstan-3-one	2.28	1.07	3.70
Androst-4-ene-3β,17β-diol	1.17	0.76	0.99

TABLE XXXIV

THE COMPARATIVE RELATIVE POTENCIES OF VARIOUS ANDROGENS INUNCTED IN CORN OIL TO THE CHICK COMB OR INJECTED IN THE SAME VEHICLE (TESTOSTERONE = 1)

Androgen	Dorfman and Dorfman (1962a) Corn oil inuncted	Dorfman and Dorfman (1962b) Corn oil injected
Androst-4-ene-3,17-dione	1.78	0.21
17β-Hydroxy-5α-androstan-3-one	1.94	3.70

TABLE XXXV

THE COMPARATIVE RELATIVE POTENCIES OF VARIOUS ANDROGENS INUNCTED IN CORN OIL TO THE CHICK COMB AND INCORPORATED IN THE FOOD (TESTOSTERONE = 1)

Androgen	Dorfman and Dorfman (1962a) Corn oil inuncted	Dorfman and Dorfman (1963) In food
Androst-4-ene-3,17-dione	1.78	0.71
17α-Methyltestosterone	1.45	0.63
19-Nortestosterone	0.72	0.76
Adrenosterone	0.02	0.32
Dehydroepiandrosterone	0.55	0.07
17α-Methylandrost-5-ene-3β,17β-diol	0.65	0.05
17β-Hydroxy-5α-androstan-3-one	1.94	0.14
Epiandrosterone	0.23	0.06
5α-Androstane-3,17-dione	0.18	0.24
11β-Hydroxyandrost-4-ene 3,17-dione	0.46	0.43

TABLE XXXVI

THE COMPARATIVE RELATIVE ACTIVITIES OF VARIOUS ANDROGENS INUNCTED IN ETHANOL TO THE CHICK COMB AND INCORPORATED IN THE FOOD (TESTOSTERONE = 1)

Androgen	Dorfman and Dorfman (1962a) Ethanol inuncted	Ofner *et el.* (1962a,b) Ethanol inuncted	Dorfman and Dorfman (1963) In food
Androst-4-ene-3,17-dione	1.21	2.60	0.71
17α-Methyltestosterone	1.86	2.31	0.63
19-Nortestosterone		0.86	0.76
Adrenosterone		0.29	0.32
Dehydroepiandrosterone	0.59	0.66	0.07
17α-Methylandrost-5-ene-3β,17β-diol	-	0.87	0.05
17β-Hydroxy-5α-androstan-3-one	2.28	1.07	0.14
Epiandrosterone	–	0.67	0.06
5α-Androstane-3,17-dione	1.15	1.82	0.24
11β-Hydroxyandrost-4-ene-3,17-dione	—	0.08	0.43
Androst-5-ene-3β,17β-diol	1.17	0.76	1.24

TABLE XXXVII

The Comparative Relative Comb Growth Activity of Androgens Injected in Corn Oil or Incorporated in the Food (Testosterone = 1)

Androgen	Dorfman and Dorfman (1962b) Corn oil injected	Dorfman and Dorfman (1963) In food
Androst-4-ene-3,17-dione	0.21	0.71
17β-Hydroxy-5α-androstan-3-one	3.70	0.14
Androst-4-ene-3α,17β-diol	1.01	0.97
Androst-4-ene-3β,17β-diol	0.99	1.24

TABLE XXXVIII

The Influence of the Structural Change from Normal D Ring to *D*-Homo on Androgenic Activity Measured by a Capon Comb Assay Method[a]

Steroid	Change in potency from *D*-normal to *D*-homo (%)
Androsterone	None
Epiandrosterone	510
5α-Androstane-3,17-dione	130
5α-Androstane-3β,17β-diol	310
5α-Androstane-3β,17α-diol	200
17β-Hydroxy-5α-androstan-3-one	None
Testosterone	50
Epiandrosterone	50
17α-Methyltestosterone	200
17β-Methylepitestosterone	Both inactive

[a] Heusser *et al.* (1954).

TABLE XXXIX

THE RELATIVE POTENCIES OF VARIOUS ANDROGENS BY DIFFERENT INJECTION AND INUNCTION TESTS IN THE CAPON (TESTOSTERONE = 1)

	Injection tests		Inunction tests	
Androgen	Tschopp (1935) Ruzicka *et al.* (1934)	Butenandt and Tscherning (1934)	Fussganger (1934) Voss (1937)	Dessau (1935, 1937)
17β-Hydroxy-5α-androstan-3-one	1.0	0 6	—	—
Methyltestosterone	0.6	—	—	1.0
5α-Androstane-3α,17β-diol	0.6	0.6	2.0	0.5
Androsterone	0.15	0.16	0.8	1.0
Androst-4-ene-3,17-dione	0.13	0 14	2.5	1.0
5α-Androstane-3,17-dione	0.13	0.09	—	—
Androst-5-ene-3β,17β-diol	0.03	0 02	—	—
Epiandrosterone	0.02	0 02	0.1	—
5α-Androstane-3β,17β-diol	0.03	-	0.2	0.1

TABLE XL

THE COMPARATIVE RELATIVE POTENCIES OF SUBCUTANEOUSLY INJECTED ANDROGENS IN A CASTRATED RAT ASSAY USING THE SEMINAL VESICLE WEIGHT END POINT (TESTOSTERONE = 1)

Androgen	Castrated rat method Eisenberg and Gordan (1950)	Castrated rat method Hershberger *et al.* (1953)
17α-Methylandrost-5-ene-3β,17β-diol	0.15	0.19
Methyltestosterone	0.72	1.0
19-Nortestosterone	0.18	0.1

TABLE XLI

THE RELATIVE POTENCY OF VARIOUS SUBCUTANEOUSLY INJECTED ANDROGENS IN A CASTRATED RAT ASSAY PROCEDURE USING PROSTATE WEIGHT AS THE END POINT (TESTOSTERONE = 1)

Androgen	Segaloff and Gabbard (1962, 1963), Segaloff (1963)	Dorfman and Kincl (1963, 1964)	Other references
17β-Hydroxy-5α-androstan-3-one	0.19	—	—
5α-Androstane-3,17-dione	1.94	—	—
Androst-4-ene-3,17-dione	1.9	—	—
5α-Androstan-17β-ol	0.02	—	—
19-Nortestosterone	0.4	—	0.3, Wilds and Nelson (1953)
Methyltestosterone	—	1.0	—
19-Norandrost-4-ene-3,17-dione	0.1	—	0.2, Wilds and Nelson (1953)
17α-Methylandrost-5-ene-3β,17β-diol	—	—	0.2, Sala and Baldratti (1957)
17α-Methyl-19-nortestosterone	0.7	0.25	—

TABLE XLII

CHANGE IN ACTIVITY OF THE TESTOSTERONE MOLECULE UPON ESTERIFICATION OR METHYLATION (SUBCUTANEOUS INJECTION)[a]

Steroid	Relative potency (testosterone = 1) Ventral prostate	Seminal vesicles
Dichloroacetate	10.3	7.7
Fluorochloroacetate	2.3	3.6
Testosterone propionate	1.7	1.7
Testosterone acetate	1.0	0.9
17-Methylether	0.34	0.23

[a] Dorfman and Kincl (1963) and Kincl and Dorfman (1964).

TABLE XLIII

THE EFFECT OF VARIOUS SUBSTITUENTS AT CARBON-2 ON THE ANDROGENIC ACTIVITY OF THE 5α-ANDROST-2-EN-17β-OL MOLECULE (SUBCUTANEOUS INJECTION)[a]

Derivative of 5α-androst-2-en-17β-ol	Relative potency (testosterone = 1) androgenic activity
2-Methyl-	1.8
2-Nitrilo-	1.5
2-Hydroxymethyl-	0.6
2-Fluoromethyl-	0.5
2-Formyl-	0.1
2-Carboxy-(17-acetate)	0.1
2-Difluoromethyl-	0.06
2-Hydroxyethyl-	0.05

[a] Dorfman and Kincl (1963) and Kincl and Dorfman (1964).

TABLE XLIV

THE EFFECT OF THE INTRODUCTION OF UNSATURATION ON ANDROGENIC ACTIVITY OF THE 2-SUBSTITUTED 5α-ANDROSTAN-17β-OL MOLECULE (SUBCUTANEOUS INJECTION)[a]

Derivatives of 5α-androstan-17β-ol	Relative potency (testosterone = 1) androgenic activity
2-Methyl-Δ^2	1.8
2-Methyl-Δ^2[b]	0.36
2-Methyl-Δ^1[b]	0.26
2α-Formyl-	0.1
2-Formyl-Δ^2	0.1
2-Methylene[b]	0.07
2-Formyl-$\Delta^{2,4}$[b]	0.06

[a] Dorfman and Kincl (1963) and Kincl and Dorfman (1964).

[b] As the 17-acetate.

TABLE XLV

THE EFFECT OF UNSATURATION ON THE ANDROGENIC ACTIVITY OF THE 5α-ANDROSTAN-17β-OL MOLECULE (SUBCUTANEOUS INJECTION)[a]

Derivatives of 5α-androstan-17β-ol	Relative potency (testosterone = 1) androgenic activity
Δ^{2b}	0.6
Δ^{1}	0.6
$\Delta^{1,3}$	0.4
Δ^{3}	0.4
Δ^{4b}	0.2

[a] Dorfman and Kincl (1963) and Kincl and Dorfman (1964).
[b] As the 17-acetate.

TABLE XLVI

THE EFFECT OF THE INTRODUCTION OF THE 17α-METHYL GROUP ON THE ANDROGENIC ACTIVITY OF 2-SUBSTITUTED-5α-ANDROSTAN-17β-OL DERIVATIVES (SUBCUTANEOUS INJECTION)[a]

Derivatives of 5α-androstan-17β-ol	Relative potency (testosterone = 1)	
	17α-H androgenic	17α-Methyl androgenic
2-Methyl-Δ^{2}	1.8	0.75
Δ^{2}	0.6	0.80
2α,3α-Difluoromethylene	0.35	0.28
2-Formyl-Δ^{2}	0.1	0.36
2-Methylene	0.07	0.38

[a] Dorfman and Kincl (1963) and Kincl and Dorfman (1964).

TABLE XLVII

THE EFFECT OF C–10 SUBSTITUTION ON THE ANDROGENIC ACTIVITY OF 19-NORTESTOSTERONE (SUBCUTANEOUS INJECTION)[a,b]

Derivatives of 19-nortestosterone	Relative potency (testosterone = 1) androgenic activity
None	0.06[b]
10-Ethyl-	0.04
10-Vinyl-	0.82

[a] Kincl and Dorfman (1964).
[b] Saunders and Drill (1956).

TABLE XLVIII

THE EFFECTS OF VARIOUS SUBSTITUENTS ON THE 17α-METHYL-5α-ANDROSTAN 17β-OL MOLECULE (GAVAGE)[a]

Derivatives of 17α-methyl-5α-androstan-17β-ol	Relative potency (methyltestosterone = 1) androgenic activity
Δ^2	1.7
2-Nitrilo-Δ^2	1.3
2-Methylene	1.1
3-Methylene	0.58
2-Carboxy	0.33
2-Formyl	0.23

[a] Kincl and Dorfman (1964).

TABLE XLIX

THE INFLUENCE OF THE 7α-METHYL GROUP ON THE ANDROGENICITY OF VARIOUS STEROIDS AS MEASURED BY VARIOUS TESTS[a]

Parent steroid	Influence of 7α-methyl group—% activity of parent steroid				
		Castrate rat, gavage		Castrate rat, injection	
	Chick comb inunction	Seminal vesicles	Ventral prostate	Seminal vesicles	Ventral prostate
19-Nortestosterone	100	400	1400	600	300
Testosterone	100	100	100	100	800
Androst-4-ene-3,17-dione	13	100	25	50	5
19-Norandrost-4-ene-3,17-dione	100	200	1900	100	170
17α-Methyltestosterone	1000	200[b] 200[b]	100	400	100
17α-Methyl-19-nortestosterone	1400	1800[b] 15000[b]	1800	10000	6000

[a] Segaloff (1963).
[b] Campbell *et al.* (1963).

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